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Cover: The six cover photographs are all related to the article on least terns in this issue. Top row: Adult least terns and the Cane Ridge Wildlife Area adjacent to the Gibson Generating Station. Middle row: Ring-billed gulls eating eggs of the least tern. Bottom row: Coyote—one of many vertebrate predators—at the least tern nesting site; and a least tern egg and chick (about a day old). (Photo of adult least terns and aerial photo of Gibson Generating Station by Ron Austing of Cinergy; other photos by Travis DeVault.)

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USE OF CHITINASE AND CHITOSANASE TO FORM SPHAEROPLASTS FROM *PILOBOLUS* SPORANGIOSPORES

Tara J. Faber and **K. Michael Foos**: Department of Biology, Indiana University East, Richmond, Indiana 47374 USA

Donald G. Ruch: Department of Biology, Ball State University, Muncie, Indiana 47306 USA

ABSTRACT. Extracting cell contents, such as DNA and protein, from cells with cell walls creates a special challenge because the wall has to be removed without damaging the contents of the cell itself. One method to remove cell walls is to use enzymes to break down cell wall components to release the cell's sphaeroplast. This study was undertaken to develop a technique that could provide sphaeroplasts from sporangiospores of the zygomycete, *Pilobolus*. Sphaeroplasts from some fungi have been prepared by digesting the germ tube cell wall more successfully than digesting the spore wall itself. However, sphaeroplasts were released more readily from ungerminated *Pilobolus* sporangiospores than from the germ tubes growing from spores. An enzyme suspension containing a combination of chitinase and chitosanase readily produced intact sphaeroplasts from which sufficient DNA could be extracted to do sequencing studies.

Keywords: *Pilobolus*, chitinase, chitosanase, sphaeroplasts, zygomycetes

Molecular techniques are central to understanding many aspects of biology and can be used to identify the various taxa and to study the relationships of a wide range of organisms. Cell contents, whether DNA, RNA or protein can be extracted from cells and purified. Once nucleic acids or proteins have been extracted, many molecular techniques can be employed and the literature is replete with techniques that have been used with specific organisms. However, it is particularly difficult to obtain cellular contents from cells with resistant cell walls.

Mycelia are used frequently as the source of fungal cell components because hyphal cell walls are often weaker than spore cell walls (Lalithakumari 2000). However, use of hyphal cells requires fungal growth before the cellular contents can be obtained. It has been reported that isolates of *Pilobolus* cannot be cultivated on agar (Gronvold et al. 2004). While that is often the case, some isolates can be maintained in the laboratory, but the majority of isolates of *Pilobolus* cannot be cultured on laboratory media and are lost before they can be examined. When isolates that cannot be cultured are lost, we can never know whether they were isolates of taxa studied previously, or whether they are new taxa that have never been collected. If cellular components could be obtained from spores of individual sporangia, it would not be necessary to culture the fungus. For this reason, the focus of this work was to develop a technique to use spores collected from the sporangia of *Pilobolus* growing on natural media as the source of cellular material.

Sporangiospores of *Pilobolus* are contained within sporangia, making the sporangium the repository of a sample of cells that can be a source of DNA or protein. However, these cells are covered with rigid cell walls that must be broken to obtain cellular contents. Use of enzymes to degrade the ungerminated sporangiospore cell walls, or of the cell walls of germ tubes from germinating spores, appeared to be one of the methods of choice.

The literature revealed a range of protocols using enzymes to degrade fungal cell walls (Binding & Weber 1974, 1978; Bon et al. 2000; Cheng & Belanger 2000; Conway et al. 2001; Petit & Arveiler 1994). However, most techniques describe sphaeroplast formation in basidiomycetes or ascomycetes (Lalithakumari 2000).Little work has been done with zygomycetes and an extensive search of the literature revealed no protocols for removing *Pilobolus* sporangiospore cell walls. We fo-

cused on the work of Binding & Weber (1974, 1978) since their work was with *Phycomyces*, a zygomycete, and tried several variations with *Pilobolus*.

While the chemical nature of *Pilobolus* sporangiospore cell walls has not been reported, it is widely assumed that, as with other zygomycetes, they are comprised of some combination of chitin and/or chitosan. Thus, we attempted to use chitinase and chitosanase to degrade sporangiospore cell walls.

METHODS

Isolates and culturing of *Pilobolus*.—Four isolates of *Pilobolus*, three of *P. kleinii* and one of *P. crystallinus*, were used in this study. *Pilobolus crystallinus* and two isolates of *P. kleinii* were obtained from herbivores in Yellowstone National Park; the third isolate of *P. kleinii* was from cattle from southern New Mexico. These isolates were maintained at ambient temperature of 22 °C ± 2 °C, with alternating 12 h light and dark periods of 2000 lux cool white fluorescent illumination. Isolates were cultured on a synthetic hemin medium (SHM) (Levetin & Caroselli 1976) in disposable plastic Petri dishes sealed with Parafilm®. Upon discharge, the sporangia of *Pilobolus* adhered to the lids of the Petri dishes and were collected for enzyme experiments using a sterile inoculating loop.

Enzyme studies.—A solution of 1% chitosanase was made by adding 16.3 ml of 0.1 M phosphate buffer at pH 5 to the tube containing 0.163 mg of lyophilized chitosanase (Sigma C-9830). A solution of 2% chitinase was made by adding 0.3 mg powdered chitinase (Sigma C-7809) to 15 ml of 0.1 M phosphate buffer at pH 6.

Solutions containing both enzymes were prepared by adding 25 µl of 1% chitosanase solution and 25 µl of 2% chitinase solution to 50 µl of 3.0 M sorbitol containing 1% Tween 20®. This resulted in a working solution of 0.25% chitosanase and 0.5% chitinase in 1.5 M sorbitol with 0.5% Tween 20®. The mixture of both enzymes together (pH 5.5, 30 °C) was used to degrade the spore walls. All treatment solutions contained 1.5 M to 3.2 M sorbitol which reduced cytolysis of sphaeroplasts, and Tween 20® to reduce sporangiospore clumping.

Multiple enzyme treatments were attempted both with germinated and ungerminated sporangiospores. Chitinase and chitosanase were used individually at their optimum pH values and temperatures. Then, both enzymes were used sequentially. Chitinase (pH 6, 24 °C) was used before chitosanase (pH 5, 37 °C), and chitosanase was used before chitinase to degrade spore walls.

Germinated sporangiospores were produced by introducing 8–10 sporangia from a Petri dish lid into a solution of 50% SHM and 50% water and incubating the spores at 37 °C for 24 h and then at room temperature for 24 h.

Ungerminated sporangiospores were used to produce sphaeroplasts by introducing 8–10 sporangia, collected from a Petri dish lid, to a 1.5 ml micro-centrifuge tube containing 100 µl of one of the enzyme solution regimens listed above. This was done for all four isolates. The tubes were vortexed for 10 sec and incubated at 30 °C for 4 h. Following incubation, 15 µl samples of spore suspension were removed and examined for sphaeroplast formation using brightfield, phase contrast, fluorescent, or Huffman modulated interference contrast microscopy. Fluorescent staining, using calcofluor white (Harrington & Hageage 1991) made empty cell walls of the

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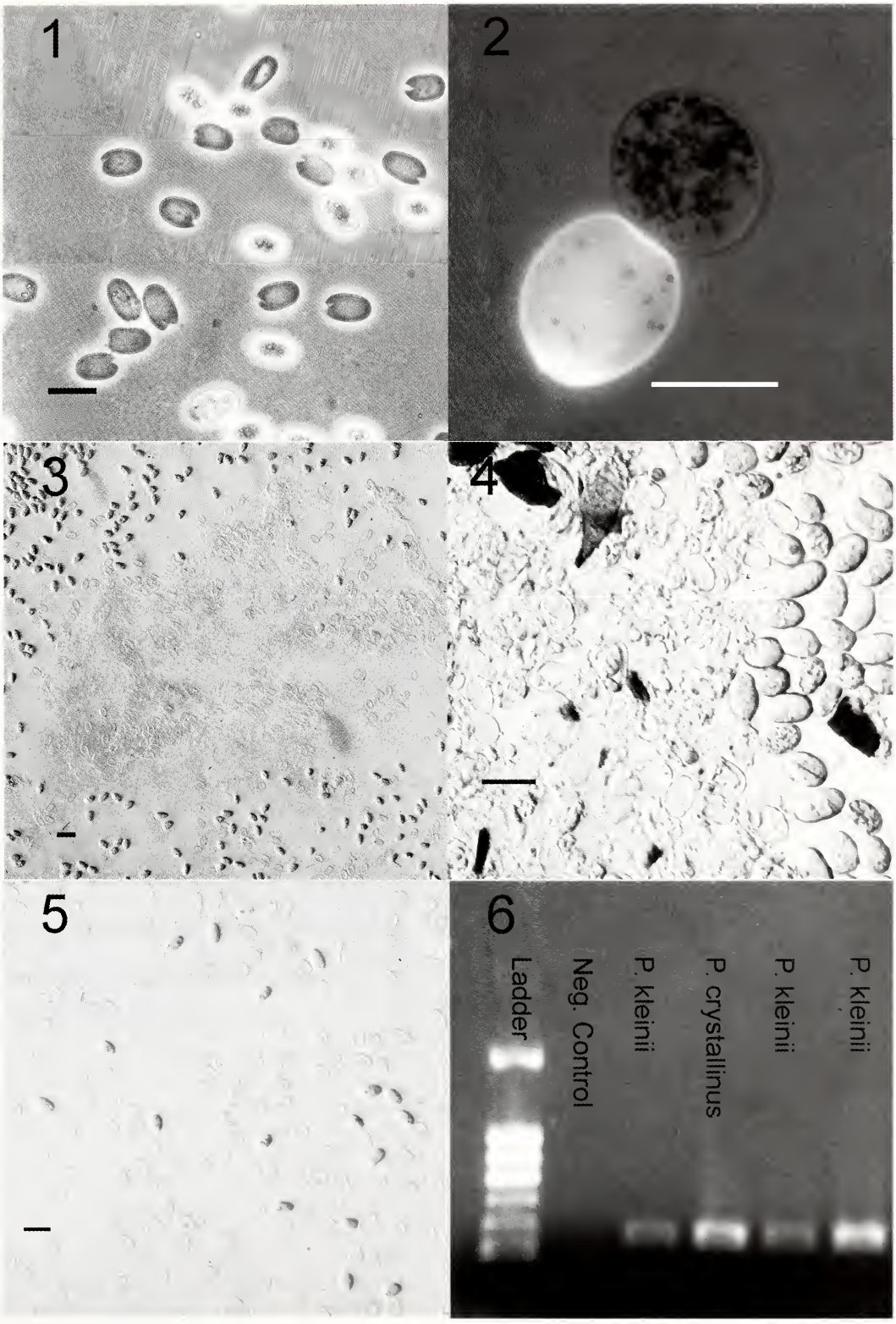
Figures 1–6.—1. *Pilobolus crystallinus*, 200×. Phase contrast microscopy shows empty sporangiospore cell walls and intact sporangiospores. Note the fissures near the ends of the spores when sphaeroplasts were released. Scale = 10 µm; 2. *Pilobolus kleinii*, 500×. Fluorescent microscopy shows a single sphaeroplast emerging from sporangiospore stained with 1% calcofluor white. Scale = 10 µm; 3. *Pilobolus kleinii*, 50×. Huffman modulated interference contrast microscopy shows degraded spore walls surrounded by intact spores. Scale = 10 µm; 4. *Pilobolus kleinii*, 200×. Huffman modulated interference contrast microscopy shows boundary between sporangiospores cell walls (left) and intact sporangiospores (right). Scale = 10 µm; 5. *Pilobolus kleinii*, 100×. Huffman modulated interference contrast microscopy shows a sample of spores after enzyme treatment. Over 70% of the spores in this field of view were lysed. Scale = 10 µm; 6. Agarose gel electrophoresis of amplified DNA from *Pilobolus* sporangiospores shows bands of approximately 520 bp fragments of DNA.


1
2
3
4
5
6
Ladder
Neg. Control
P. kleinii
P. crystallinus
P. kleinii
P. kleinii

spores show up particularly well. Images were captured on 35 mm Kodachrome® 64 film and digitized using a Nikon Coolscan® V-ED 35mm Film Scanner. Adobe Photoshop Elements® was used to crop, insert scale, remove color and sharpen images.

DNA isolation and study.—A suspension of sporangiospores that had been lysed using the combined enzyme solution was washed twice with pH 5.5 phosphate buffer, centrifuged for 5 min at 3000 rpm, and added to tubes of a MoBio UltraClean® Soil DNA kit. The initial steps in the protocol requiring mechanical cell disruption were omitted, and the DNA purification steps of the protocol were followed. DNA was stored in water. Samples of DNA were amplified using AmpliTaq® Gold DNA polymerase (Applied Biosystems, Foster City, California) and a dNTP mix (Promega Corporation, Fitchburg, Wisconsin). Thermal cycling was conducted in an Elmer Perkin GeneAmp® PCR System 2400. Primer sets NS1 and NS2 used in these experiments were from White et al. (1990). Reaction conditions for thermal cycling were 95 °C for 5 min, followed by 36 cycles of 95 °C for 1 min, 55 °C for 1 min 30 sec, 72 °C for 1 min 30 sec, followed by an extension at 72 °C for 10 min. PCR products were subsequently purified using the QIAquick® PCR Purification Kit (Qiagen, Inc., Valencia, California).

PCR-amplified DNA fragments were electrophoresed, prior to and following the clean-up process, on 1% agarose gels in 1× TBE buffer (50 mM Tris-HCl, 50 mM boric acid, and 1 mM EDTA) containing ethidium bromide, and visualized using a ChemiImager® 4400 Imaging System (Alpha Innotech, San Leandro, California). A 100 bp DNA ladder (Takara Mirus Bio, Madison, Wisconsin) was used as a size marker.

The PCR products were sequenced using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California) and a sequence reaction mix comprised of 2 μl H_2O, 3 μl 5× buffer, 1 μl BigDye, 2 μl 10 mM primer, and 2 μl DNA. Thermal cycling conditions for sequencing were 25 cycles of 96 °C for 10 sec, 50 °C for 5 sec and 60 °C for 4 min. Sequences were analyzed using an Applied Biosystems 3700 automated fluorescence system.

RESULTS

Initial studies compared the use of germ tubes of germinating spores, and ungerminated spores, as sources of sphaeroplasts. The germinated spores were exposed to several different enzyme regimens. They were introduced into (1) chitosanase alone, (2) chitinase alone, (3) chitinase followed by chitosanase, (4) chitosanase followed by chitinase, and (5) a mixture of chitinase and chitosanase. Length of treatment varied from 2–34 h.

All five enzyme treatments of germinated sporangiospores resulted in production of sphaeroplasts. However, microscopic examination of spores treated by all of these techniques showed that most spores had not ruptured. However, the mixture of chitinase and chitosanase worked as well as, but generally better, than the other treatments.

The ungerminated spores were exposed to the same enzyme regimens. Treatments with a single enzyme at the enzyme optimum temperatures and pH caused cell lysis with the release of many sphaeroplasts; however, large numbers of intact spores remained. Likewise, treatments using the two enzymes, sequentially, at their optimum temperatures and pH values, in either order, produced some sphaeroplasts, but left many intact spores. However, treatments using a mixture of chitinase and chitosanase together at 30 °C and pH 5.5 produced a higher proportion of sphaeroplasts than any other regimen of enzymes.

Microscopic techniques were used to determine whether or not sporangiospore cell walls were being degraded with the release of sphaeroplasts as a result of enzyme treatments. Brightfield microscopic views provided very little contrast between sporangiospores that were intact and those that had been degraded by the enzyme regimens. Phase contrast images of sporangiospore cell walls devoid of contents can readily be distinguished from intact sporangiospores. Many spore walls exhibit fissures near the ends of the spores (Fig. 1). Calcofluor white staining caused spore cell walls to fluoresce. Figure 2 shows a single sphaeroplast emerging from its fluorescing spore wall stained with calcofluor white. The sphaeroplast is readily visible, but much darker that the fluorescing spore wall.

Distribution of sporangiospores within the enzyme solution can be seen by preparing wet

mount slides of this material. Figure 3 shows such a wet mount demonstrating enzyme action on the cells near the center of the field. Spores near the periphery of the field of view remain intact.

Because *Pilobolus* sporangiospores are embedded in a common matrix, they tend to clump or stick together. Within a cluster of spores, the spore walls are degraded when acted upon by enzymes. These spores are the ones near the periphery of the clump. Where the enzymes do not penetrate the clumps, spores remain intact. Figure 4 shows an area in which enzymes acted upon many spores, whereas other spores remain intact. There is a distinct border between spores with enzyme degraded walls (left) and those remaining intact (right).

As clumps of spores are broken apart, those with intact cell walls can be distinguished from the empty cell walls remaining from the disrupted spores. Huffman modulated interference contrast microscopy shows the outlines of empty sporangiospore cell walls as distinct from intact spores (Fig. 5).

After determining microscopically that sphaeroplasts were being formed, it was necessary to determine whether or not DNA from the sphaeroplasts could be used for molecular studies. Gel electrophoresis of DNA prior to amplification showed no bands, however after PCR amplification DNA from these sphaeroplasts showed strong bands of 520 bp fragments of DNA from each of the isolates (Fig. 6). When these DNA fragments were sequenced, analyzed, and compared with other sequences, they were consistent with similar fungi from GenBank. The fragments were deposited in GenBank with accession numbers DQ363379–DQ363382.

DISCUSSION

All of the enzyme treatments produced some sphaeroplasts from sporangiospores that had been disrupted. With each treatment some empty cell walls were observed, other spores remained intact. Nearly all of the sporangiospores were broken in some samples, whereas others contained fewer disrupted spores. Treatments utilizing multiple enzyme treatments, with different conditions of temperatures and pH, were more labor intensive and less productive than a combined treatment. Although we did not count the number of intact and disrupted spores, it was clear that a combination of chitinase and chitosanase worked very well to release sphaeroplasts from *Pilobolus* sporangiospores. Relatively small amounts of DNA were released from these spores; however, there was sufficient DNA after PCR amplification to sequence the fragments for comparative study.

It appeared that the disruption of particular cells by enzyme activity was related to the distribution of the sporangiospores within the enzyme suspension. *Pilobolus* sporangiospores adhere to each other in a common matrix. Within the sporangium, an adhesive matrix causes the several thousand sporangiospores to form a multicellular mass. This matrix causes the sporangium to stick to herbaceous material when discharged, but makes it difficult to treat the individual spores with enzymes. Tween 20® was used to reduce adhesion within the matrix; yet many spores adhered as a mass. The regimen of enzymes appeared to disrupt spore cell walls upon contact with the surface of the spore wall. Thus, regardless of the effectiveness of the method for degrading spore cell walls, many of the spores remained intact. This inter-spore adhesion is an impediment to sphaeroplast production from *Pilobolus* sporangiospores.

Since this technique was used with multiple isolates of *Pilobolus* of two species, it should work with all species of this particular genus. It is a relatively quick, easy, straightforward technique to disrupt sporangial cell walls with the release of DNA. A major asset of this technique is that it works with relatively small numbers of cells. There are approximately 50,000 spores within a single sporangium producing sufficient DNA for sequencing. By using sporangiospores from a single sporangium to provide DNA, isolates of these fungi, which are often difficult or impossible to maintain in the laboratory, can be studied.

Of the various enzyme regimens used, we found a combination of chitinase and chitosanase worked best to remove the cell walls from *Pilobolus* sporangiospores. Because of the high proportion of lysed spores, we conclude that the mode of action is appropriate for the composition of the sporangiospore cell wall and speculate that the sporangiospore wall is comprised of, at least in large part, chitin and chitosan.

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CONTROL OF BRANCH INITIATION IN *NEUROSPORA*

Michael K. Watters: Department of Biology, Neils Science Center, Valparaiso University, 1610 Campus Drive East, Valparaiso, Indiana 46383

ABSTRACT. In a previous study of branching in *Neurospora*, it was determined that branching at the tip is not a tip autonomous process, but that branching is controlled at least in part by a factor or factors at or near the previous branch point. This was determined by the demonstration of a statistical correlation between lengths of branch intervals (the distance between two tandem branch points) having a common origin. That study was unable to determine the nature of that common factor. Namely, the correlation could have been due to the physical division of a structure or resource present at the time and location of the common branch point. It could have alternatively been caused by the division, at the common branch point, of the flow of an undetermined factor toward the growing tips. This study distinguishes between these two alternatives by extending the examination of branch interval correlation and comparing branch intervals that share a common origin, one step removed. The observation of branch length correlation at this level suggests that the previously observed correlation primarily results from the division of an undetermined factor flowing toward the tip and not the singular division of a structure at the time of branch formation.

Keywords: *Neurospora crassa*, morphology, branching, hypha

Filamentous fungi grow by hyphal tip extension and by making new hyphal tips (branching). These two processes are fundamental aspects of fungal biology and have attracted much attention in research. Of the two processes, tip growth has been more extensively studied, leading to reasonable models for the cellular mechanisms involved (Bartnicki-Garcia 1973; Collinge & Trinci 1974; Heath 1990; Howard 1981; Riquelme & Bartnicki-Garcia 2004). There are a number of mathematical models for tip growth and morphogenesis (Hutchinson et al. 1980; Kotov & Reshitnikov 1990, Yang et al. 1992), but these do not attempt to explain branch initiation, and usually assume branching to be a random event. A branch, once formed, must abide by the same growth mechanisms as the original apex. However, first a critical event presumably takes place that commits the cell to form a new branch. Among published models for branch initiation, there are two basic categories. These differ fundamentally in the proposed source of the branching signal.

The focus of one category of models is on branch initiation by factors originating proximal to the branch event itself, from somewhere within the colony. In *Neurospora crassa*, the protoplasm for tip extension can come from regions of the mycelium more than 12 mm from the colony margin (Zalokar 1959). Katz et al. (1972) proposed that a new branch would be initiated when the extension capacity of the hypha overcomes the extension capacity of existing tips. Trinci (1974) and Prosser & Trinci (1979) proposed that tip growth vesicles are the key elements whose accumulation triggers branching.

In the second category of branching models, the focus is on events that are controlled independently at the tip. Bartnicki-Garcia et al. (1989a, b) suggested that there might also be a tip-based pulling mechanism that displaces the vesicle supply center (VSC) for branching. Possible molecular candidates for this role are microfilaments (Bartnicki-Garcia et al. 1989a, b), integrin (Kaminskyj & Heath 1996) and spectrin (Degousee et al. 1997). Calcium has been invoked as a factor important in tip growth and branching (Heath 1990). A cytoplasmic Ca^{2+} gradient is thought to play a role in maintaining apical dominance by inhibiting branch formation at the apex of a hyphal tip (Schmid & Harold 1988). This idea is supported by the work of Reissig & Kinney (1983), Prokisch et al. (1997), Capelli et al. (1997), Levina et al. (1995) and Grinberg & Heath (1997).

Parallel to the question of control of branching generally, is the issue of lateral *vs.*

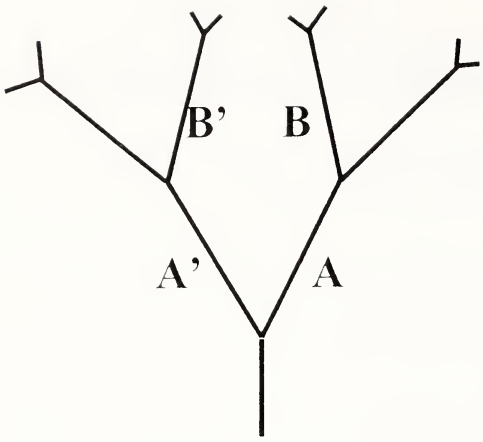

Figure 1.—Definition of branch interval pairs discussed in the text. A branch interval is defined as the distance between tandem two branch points. The pairs of intervals discussed in the text either compare intervals having a common origin (A *vs.* A′) or those who immediately follow intervals having a common origin (B *vs.* B′). For the purpose of this study, the B and B′ intervals were defined either as those intervals occurring along the main hypha (for lateral branches) or the central two branch intervals (for apical branches).

apical branching. It is unclear at present if the two morphological forms are simply variations of a single process or if they represent the results of fundamentally different events. Observations of Spitzenkörper behavior by Riquelme & Barnicki-Garcia (2004) show very different behavior during apical and lateral branch formation. In addition, growth can be induced to switch from lateral to apical branching by either mutation or environmental insult (Scott 1976; Watters et al. 2000a; Watters & Griffiths 2001). These lines of evidence suggest lateral and apical branch points are fundamentally distinct from each other. However, statistical examination of branching (Watters et al. 2000a, b; Watters & Griffiths 2001) has suggested that apical and lateral branching share much of their control, with apical and lateral branch arrays both showing symmetry (branch intervals having a common origin show correlation for their lengths) and similar distributions (in terms of the shape of the curve, but not the means) of spacing between branch points.

Watters et al. (2000b) demonstrated that the lengths of branch intervals sharing a common origin (i.e., A *vs.* A′ in Fig. 1) are correlated for their lengths. This demonstrates that although branching takes place at the tip, the decision to form a branch is not controlled independently by the tip itself. The branch decision is instead controlled, at least in part, by a factor associated with or determined by the previous branch point. Briefly, something about a branch point controls when the next branch point will form along a growing hypha. There are two alternative models that could explain the observed correlation and explain this control.

Under the first suggested model, tip growth and branching is purely under the control of a structure or resource, such as the Spitzenkörper located at the tip of a growing hypha. The Spitzenkörper is a collection of tip growth vesicles located at the tip that has been shown to play a critical role in both tip growth and branching (Bartnicki-Garcia 2002; Riquelme & Bartnicki-Garcia 2004). This model is consistent with the suggestion that branching is controlled directly by the Spitzenkörper and its collection of tip growth vesicles. The degree of symmetry following a given branch event would then be proposed to be controlled by the degree of symmetry by which the resources of the Spitzenkörper are divided during a branch event.

Under the second suggested model, branching is proposed to be triggered by either a signal or by the flow of resources from the colony to the growing tip. While this could take the form of a more direct signal, it is also consistent with previously suggested models (Katz et al. 1972; Watters & Griffiths 2001) where branching is triggered by the accumulation of factors fed by the colony to the tip. These factors would most likely be material important in tip growth supplied to the tip either in vesicles or simply in the cytoplasm as it streams toward the tip. This material is generated deep in the colony and is supplied to the growing tips via cytoplasmic streaming. It has been seen in *Coprinus disseminatus* that tips do not slow their extension following a branch event (Butler 1961), so it can be concluded that the supply of this material to the tip must exceed the rate of consumption. This suggests the accumulation of a reserve of tip growth material at the tip. Under this model, once the supply in this reserve reaches a set threshold, a branching event is triggered.

The two models differ in one unique pre-

diction, namely their expectation for the lengths of branch intervals following the initial intervals observed previously (i.e., B *vs.* B′ in Fig. 1). In the first model, the critical event (the division of a structure or resource, such as the Spitzenkörper) is limited in time. When the two forks emerging from the common branch point form new branches at their apexes, the correlation should be at an end. The divisions that mark these two new branch points would be expected to be independent events, so there is no reason to expect to observe correlation past this point. In the second model, however, the critical event (the division of the flow of resources at a branch point) is on-going and is thus expected to continue to exert an influence on growth (and branching) in subsequent branching events.

In the present work, we have tried to distinguish between these two basic models by extending the study of branch correlation. Here, we examine the correlation between branch intervals that do not share a common origin, but those where the intervals immediately prior to those under consideration share a common origin. If branching is controlled solely by a resource or structure that is divided at the time of a branch event, the intervals under comparison result from independent divisions and thus should not be correlated. If, however, branching is controlled by resources flowing to the tip from deeper in the colony that are divided at branch points, the common branch point should still exert an influence and result in detectable correlation. The results presented below support the suggestion that branch symmetry results from a roughly equally divided flow of material and thus that branching is triggered somehow by resources streaming to the growing hyphal tip from within the colony. This correlation is seen for both apical and lateral branch arrays, arguing in favor of the suggestion that both branch morphologies are regulated by related mechanisms.

METHODS

Strains and media.—The standard *Neurospora crassa* Oak Ridge wild-type 74-OR81-1a (FGSC #988) was used for most experiments (McCluskey 2003). Media and culturing procedures were those described in Davis & deSerres (1970), except as noted. Cold shock in *Neurospora* induces a temporary phase during which branching is exclusively apical (Watters et al. 2000a). For the study of branch symmetry involving apical branches, colonies were subjected to cold shock; wild type was grown overnight on Vogel's medium at 25 °C, then shifted to 4 °C. Measurements were made following a 24 h incubation at 4 °C.

Photomicroscopy.—Cultures were photographed on TMX400 film, using a Olympus BH-2 microscope fitted with a 35 mm camera. Branch intervals (distances between branch points) were determined by measuring rear projected negatives. These measurements were limited by: the degree of photographic enlargement, the thickness of the average hyphae and the degree to which any individual hyphae deviated from perfect linear growth. This allowed measurement of branch interval lengths to the nearest 10 μm.

Statistical analysis.—The distribution of branch intervals in most strains is markedly skewed toward the short end of the range. Consequently, normal statistics are not appropriate. Thus, the non-parametric Spearman correlation coefficient (*Rs*) was used for all comparisons. The branch data were analyzed statistically using the program SPSS (SPSS Inc., Chicago, Illinois).

RESULTS

To distinguish between the two models presented above, a survey was done of branching in *Neurospora crassa* to detect any possible second order symmetry (B *vs.* B′, Fig. 1). Lateral and apical branches were examined separately as it was previously observed (Watters et al. 2000b) that the strength of the correlation was dependent on the morphology of the branch point. The results of the current survey (Table 1) confirm the first order (A *vs.* A′, Fig. 1) correlation previously observed, and also show a clear second order (B *vs.* B′, Fig. 1) correlation present for both lateral and apical branch patterns.

DISCUSSION

The first order (A *vs.* A′, Fig. 1) symmetry observed previously (Watters et al. 2000b) was confirmed. This reinforces the previous conclusion that branching is somehow controlled by events at the previous branch point. In addition, second order (B *vs.* B′, Fig. 1) symmetry was observed for both lateral and

Table 1.—Correlation between branch interval lengths. Statsitical analysis of paired branch interval lengths confirms the previous observation of the correlation of branch interval lengths sharing a common origin (A *vs.* A′), as well as the second order (B *vs.* B′) symmetry currently under test. All correlations were observed with *P* values below 0.01. *Rs* = Spearman rank correlation coefficient, *n* = Sample size.

Branch morphology		A *vs.* A′	B *vs.* B′
Lateral branches	*Rs*	0.124	0.115
	P value	0.003	0.007
	n	549	549
Apical branches	*Rs*	0.686	0.585
	P values	<0.001	<0.001
	n	206	206

apical branch arrays. This supports the assertion that the primary cause of the observed symmetry is the division of resources flowing through branch points. The strength of the second order correlations observed parallels that observed for the first order symmetry (Watters et al. 2000b). Specifically, the symmetry is much stronger among apical branches than it is for lateral branches. This argues that the flow of resources is divided more evenly at apical branch points than it is at lateral branch points. This might not be surprising given the obvious spatial symmetry of an apical branch *vs.* a lateral branch.

The observation of symmetry relationships in both lateral and apical branches supports the argument that despite their obvious differences, they are regulated and triggered by similar mechanisms. The fact that apical branching can be induced by environmental insults (Watters et al. 2000a), genetic mutations (Scott 1976; Perkins et al. 2001) and cytoplasmic contractions (Reynaga-Peña et al. 1995; Riquelme & Bartnicki-Garcia 2004) would, however, argue they were induced via different mechanisms. This conclusion is reinforced by the direct observations of Spitzenkörper behavior during lateral and apical branch formation (Riquelme & Bartnicki-Garcia 2004). If apical and lateral branches are triggered differently, the proposed mechanisms must be able to explain how both triggering mechanisms produce the symmetry observed above and previously (Watters et al. 2000b). They must also explain the similar distributions of branch intervals observed previously (Watters et al. 2000a).

Lateral branches have previously been suggested to be induced by the accumulation of a critical factor at the tip (Katz et al. 1972; Watters & Griffiths 2001). These factors (generally suggested to be vesicles containing material for tip growth) have their origins in the colony and are fed to the tip via cytoplasmic streaming. The flow of this material would by necessity be divided at branch points. If this flow is indeed critical to the decision to form a lateral branch, it would result in the correlation observed above.

Apical branches have long been suspected to be induced by an alternative process. Riquelme & Barnicki-Garcia (2004) recently observed that apical branch formation was immediately preceded by a rapid cytoplasmic contraction that resulted in the dissolution of the Spitzenkörper followed by the reformation of a pair of Spitzenkörper forming the two tips of an apical branch. If such contractions have their origins deep enough in the colony, any contraction would propagate equally through branch points, sending this 'signal' through both hyphae emerging from a branch point to be received by growing tips at the same time. This alternative branching signal would still have its origin in the colony and show the observed symmetry, providing an alternative mechanism of control that still displayed symmetry.

Although the mean branch point to branch point distance is quite different for apical and lateral branch series, the shapes of the overall distribution of such intervals (Watters et al. 2000a) are the same. Although dual models for triggering lateral and apical branch points can both effectively produce symmetrical arrays, it is unclear, however, why such distinctive mechanisms for branch induction would produce such similarly shaped distributions of branch interval lengths.

In conclusion, the above observations demonstrate that branching is triggered by a signal(s) that has its origin in the colony. This signal flows to the tips roughly equally to result in the observed symmetry of growth. Although this symmetry is observed for both lateral and apical branches, these observations are consistent with the possibility that lateral and apical branches are induced by different signals, so long as both signals originate with-

in the colony and propagate to the tips similarly.

ACKNOWLEDGMENT

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THE HISTORY AND AQUATIC FLORA OF SILVER LAKE, PORTER COUNTY, INDIANA, WITH COMMENTS ON THE ADEQUACY OF FLORISTIC QUALITY ASSESSMENT FOR LAKES

Mitchell S. Alix and **Robin W. Scribailo**: Biology/Chemistry Department, Purdue University North Central, Westville, Indiana 46391-9542 USA

ABSTRACT. During the growing season of 2004, floristic inventories were conducted on Silver Lake, Porter County, Indiana to quantitatively describe the aquatic flora. A modified point intercept technique was used to determine the frequency and relative frequency of each taxon from a total of 41 sampling points. Measurements of physical and chemical water parameters were taken from a random subsample of these points. Floristic inventories resulted in the documentation of 42 taxa in 32 genera from 23 families. Families exhibiting the greatest richness were the Lemnaceae and Potamogetonaceae. *Nuphar advena* had the highest frequency of occurrence (>75%). Chemical and physical evidence indicates that Silver Lake is a circumneutral water body prone to acidification because of its shallow depth, poor buffering capacity, and the presence of remnant peat deposits. The floristic and natural area quality of the lake proper was evaluated using the Floristic Quality Assessment (FQA) methodology of Swink & Wilhelm (1994). The floristic quality index (I) and mean coefficient of conservatism (C_{mean}) were calculated from two sources of C values (i.e., Indiana and the Chicago region), which have overlapping geographical ranges. A comparison of C values for the Silver Lake aquatic vascular plant taxa indicated that Indiana C values were at least one unit lower than those from the Chicago region. Although the use of Indiana C values resulted in lower values of C_{mean} and I, values of C_{mean} calculated from both sets of C values indicated that Silver Lake was high in natural area quality. However, the presence of a shallow lake basin, coupled with anthropogenic disturbance and drought-induced drawdowns, has contributed to a frequent and substantial loss of littoral zone, which has negatively impacted the lake's ability to sustain floristically significant submerged aquatic taxa. We recognize that problems exist with standard FQA methodology when applied to lake ecosystems and suggest ways to improve the diagnostic resolution of the FQA metrics.

Keywords: Floristic quality assessment, Indiana flora, Silver Lake, aquatic macrophytes, C values, I_{nni}

Silver Lake is one of only two small natural lakes located inside the city limits of Valparaiso, Indiana. Much of the property surrounding the lake is under private ownership; however, the southern shore is owned by the City of Valparaiso and has been selected as a project site for a road extension. The road extension has been permitted by the United States Army Corps of Engineers and the Indiana Department of Environmental Management and will be constructed through two small wetland areas. It has been determined that this project will impact an area of <0.04 ha. Many local conservationists believe the future ecological health and floristic quality of the lake and its surrounding wetlands will be diminished, or at least compromised, by the construction and use of this thoroughfare. Unfortunately, there are limited baseline data on the quality of this lake, rendering any scientific comparison of before and after conditions almost futile. These genuine concerns provided the impetus for us to conduct this study.

The main objectives of this study were (1) to quantitatively document the aquatic macrophytes within Silver Lake, (2) to measure physical and chemical habitat parameters often used in the characterization of aquatic macrophyte communities, and (3) to determine and evaluate the floristic and natural quality of the lake proper by using the Floristic Quality Assessment (FQA) methodology developed by Swink & Wilhelm (1994). Data on the habitat parameters provided here and used in conjunction with results from the floristic analysis will serve as a benchmark for the future monitoring of Silver Lake. We also provide suggestions on how to maintain, if not enhance, the floristic quality of this Lake.

A secondary objective of the Silver Lake

analysis was to compare the Indiana coefficients of conservatism (*C* values) with those of the Chicago region (Swink & Wilhelm 1994) for the aquatic vascular plants at this site and determine which source was more appropriate for the FQA of Silver Lake. Because of its uncomplicated methodology, FQA is one of the most appealing diagnostic tools for evaluating habitat, given that one possesses sufficient taxonomic skills to correctly identify vascular plants. However, there are problems with the application of FQA to lake ecosystems. Therefore, we also examine some of the current problems with the use of FQA in the context of a lake ecosystem and provide suggestions on how to improve the adequacy of the FQA metrics.

METHODS

Study site.—Silver Lake is located in the northern portion of the City of Valparaiso in Porter County, Indiana (41°29′43″N, 87°03′34″W) directly northeast of Valparaiso High School and is situated between Campbell Street and Valparaiso Street (Fig. 1). This body of water is extremely shallow (*ca.* mean depth 0.6 m) and small (*ca.* 10.5 ha) and is the southern most lake of 13 lakes found in the Valparaiso Lakes Area watershed, which expands across 1085 ha. Much of the surface area of Silver Lake is dominated by spatterdock (*Nuphar advena*) and the majority of its shoreline is composed of cat-tails, sedges, and smartweeds. Due to its small size, shallow depth, and extensive emergent zone, Silver Lake is more reminiscent of a basin marsh than a lake. Although much of its shoreline is privately owned and no public access currently exists on this lake, the property adjacent to its south shore is owned by the City of Valparaiso and has been selected as the site for the extension of Vale Park Road to Campbell Street (Fig. 1).

Silver Lake lies within the Valparaiso Moraine Section of the Northwestern Morainal Natural Region (Homoya et al. 1985). This natural region is known for its dunes, glacial ridges, and diversity of wetland types (Post 1997). The Valparaiso Moraine Section topographically divides the Lake Michigan basin from the Kankakee River basin in northwest Indiana. Water from Silver Lake drains from an outlet located on its southeast corner (Fig. 1) to a ditch and water control structure, and then travels east through a ditch perpendicular and beneath Valparaiso Street, proceeding northward to Flint Lake, the largest lake in the Valparaiso Lakes Area watershed.

Silver Lake is found in Soil Region 7 characterized by the Blount-Morely-Pewamo association (Franzmeier 1997). These soil types are plentiful in the Valaparaiso Lakes Area watershed and were primarily formed from clayey glacial till after the retreat of the Wisconsinan glacier. With the exception of the western shore, which consists of Morely silt loam, much of the soil surrounding the immediate portions of Silver Lake, including the project site for the road extension, is Pewamo silty clay loam (Furr 1981). This very poorly-drained soil is commonly found in depressional areas and till plains in the Valparaiso Moraine Section. According to Furr (1981), this soil has severe limitations for building sites and roads because of its moderately slow permeability, which contributes to the accumulation of surface waters and its susceptibility to frost action potential; however, it is well-suited for water-tolerant vegetation.

The sediments of Silver Lake are composed of muck along the near shore areas, though remnants of peat occur in a few isolated areas especially toward the center of the lake. According to Joe Clifford (pers. comm.), a resident and property owner of land bordering the lake since the 1950s, Silver Lake was once an oak-hickory forest, having trees with diameter at breast heights (DBHs) of up to approximately 60 cm and an understory dominated by sassafras. Aerial photography from 1938 clearly shows that the current lake basin was a forested area (Fig. 2). In early November of 1953, the peat and sphagnum subsurface was ignited from a fire used to burn brush and debris from the clearing of land on the west side of the lake (Anonymous 1953a, 1953b, 1953c, 1953d). The peat burned down to the clay bottom of the original lake basin (Fig. 3) to a maximum depth of approximately 1 m, exposing the roots of the trees, which subsequently toppled over. Although some of the tree stumps and logs remain in the lake to this day, many were hauled out and chopped into firewood. The ultimate effect of this fire was to expose what likely represents the original post-glacial clay-lined lake basin. This basin, like many of those found in the northern United States, was probably originally formed as

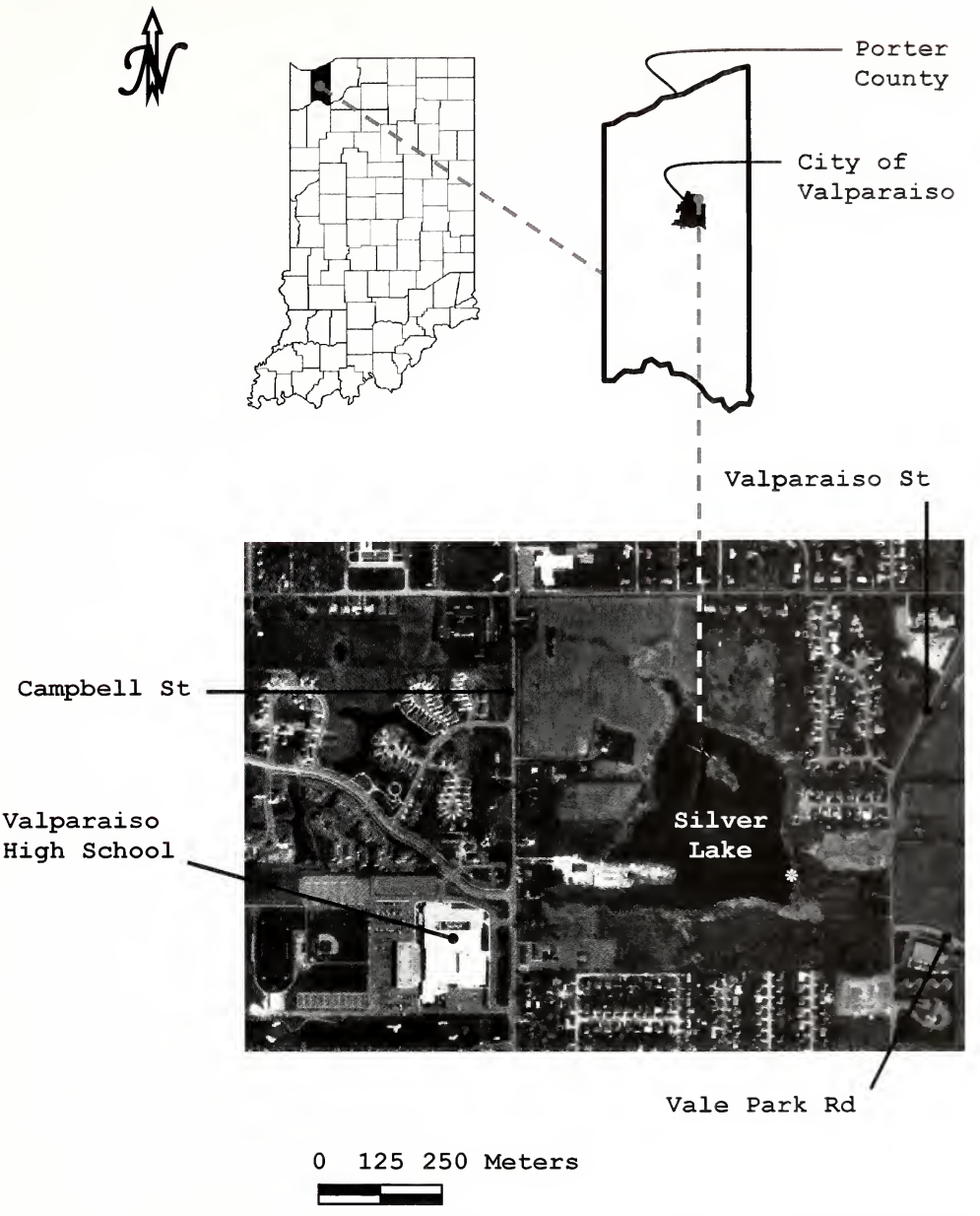
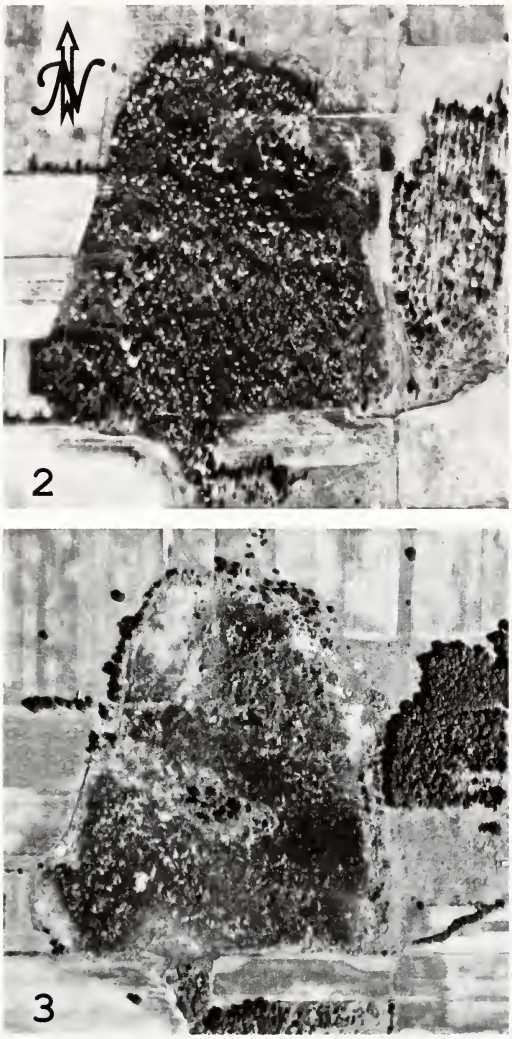


Figure 1.—Silver Lake, located in the City of Valparaiso in Porter County, Indiana. The white star at the southeast corner of the lake indicates the location of the outlet. (Orthophotograph courtesy of the United States Geological Survey.)

an ice block depression, which subsequently became a kettle hole lake following the last Wisconsinan glacial retreat. Although we believe it is not possible to accurately reconstruct the vegetational history of Silver Lake because of the loss of peat layers due to the fire, it is likely that the lake went through typical bog successional stages. It is somewhat ironic that the lake appears to have reverted back to earlier successional stages as a result of anthropogenic activity. Over the last 50 years, Silver Lake has slowly accumulated silt from runoff and organic matter from the growth of aquatic macrophytes, and is now largely covered by spatterdock.

Floristic survey and assessment.—In



Figures 2, 3.—Orthophotographs of Silver Lake area. 2. Pre-burn forested area in 1938; 3. Post-burn Silver Lake basin in 1954. (Orthophotographs courtesy of the United States Geological Survey.)

2004, field surveys were conducted during the months of May–August to inventory the aquatic flora of Silver Lake. One survey was made each month to minimize temporal effects and to ensure that diagnostic characters, such as flowers and fruits, were present for positive identifications of specimens. Areas were surveyed using a point intercept method modified from Madsen (1999), which consisted of recording the presence of species in a radial area encircling a pre-selected waypoint. This method utilized a randomly selected point of origin for the construction of a grid. The grid consisted of 41 geo-referenced waypoints having an interval of 50 m that was generated in the Universal Trans Mercator (UTM) coordinate system North American Datum 1983 (NAD 83) using Trimble TerrsSync™ software (Fig. 4). The use of 50 m grid intervals provided adequate coverage for surveying Silver Lake because of its small size, almost uniform shallow depth, and relative homogeneous macrophyte community structure. This grid and a digitized base map of Silver Lake were transferred onto a Trimble GeoXT™ global positioning system (GPS) unit outfitted with a Trimble Beacon-on-a-Belt (BoB™) real-time differential corrected receiver, which was used to navigate from point to point and record data.

At each waypoint, an area of 3 m^2 was surveyed for the occurrence of aquatic macrophytes. In areas having a depth $\leq$0.5 m, submersed, free-floating, and emergent macrophytes were collected by hand. At depths >0.5 m, an extendable thatching rake was used to collect macrophytes from the side of a kayak. The frequency of occurrence was calculated for each taxon and represents the percentage of survey areas from which a given taxon was present. Each percentage corresponds to a specific frequency category based on a scale modified from Alix & Scribailo (1998). Frequency categories for taxa are defined as follows: rare = occurrence at $\leq$10% of the total number of survey areas; infrequent = occurrence at >10%, but <20% of the total number of survey areas; occasional = occurrence between 20–50% of the total number of survey areas; common = occurrence at >50%, but <75% of the total number of survey areas; abundant = occurrence at $\geq$75% of the total number of survey areas. Each taxon was assigned to its corresponding frequency category. Frequency values were also converted to relative frequencies by dividing the frequency of an individual taxon by the sum frequency for all taxa. The frequency of occurrence, frequency category, and relative frequency of each taxon are provided in the Appendix.

The floristic quality of the lake was evaluated using Swink & Wilhelm's (1994) floristic quality index (*I*), which uses coefficients of conservatism (*C* values) of native vascular plant taxa in its calculation. A *C* value assigned to a native vascular plant taxon is rep-

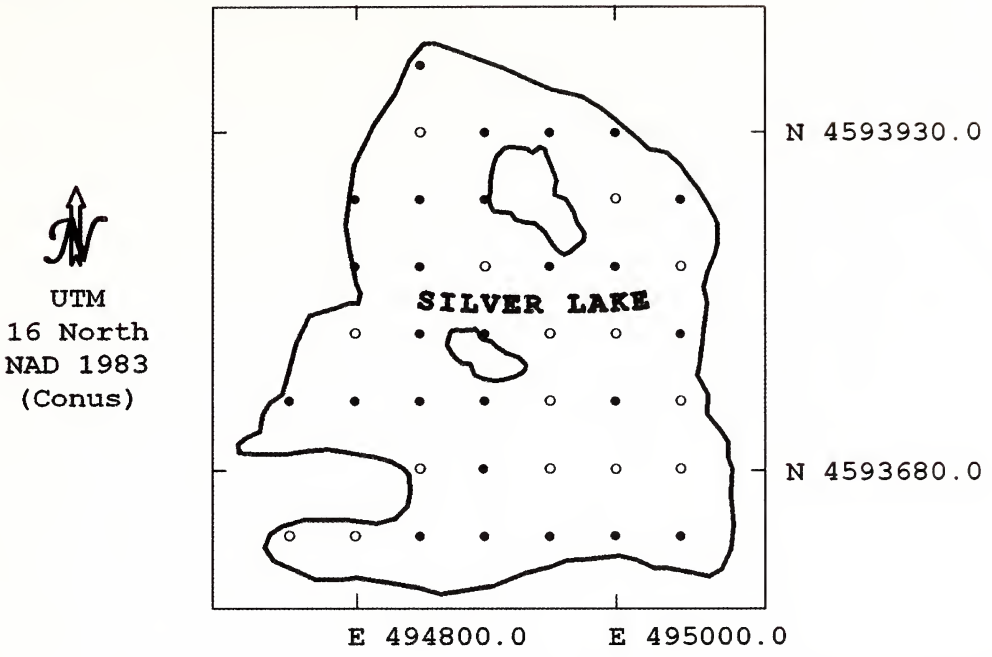


Figure 4.—Base map of Silver Lake with sampling grid. All circles (● and ○) represent point intercept survey areas. Open circles (white with black margins—○) represent sites sampled for physical and chemical parameters.

resented by an integral value ranging from 0 to 10. Each integral value theoretically represents an estimated level of fidelity to an area that has remained relatively unaltered from pre-settlement conditions (Swink & Wilhelm 1994). Taxa having a high *C* value often appear to be very sensitive to habitat degradation and show fidelity to undisturbed natural areas, whereas taxa having a low *C* value show little or no fidelity to specific natural communities and can be quite resistant to environmental disturbance. Typically, *C* values are subjectively assigned to plant taxa of a given region or political boundary based on the judgments made by a committee of professional botanists familiar with particular attributes of their behavior, such as sensitivity to disturbance and patterns of occurrence, independently of their rarity (see Swink & Wilhelm 1994; Andreas & Lichvar 1995; Herman et al. 1996; Taft et al. 1997; Nichols 1999; Rothrock 2004; Rothrock & Homoya 2005).

Because Porter County was one of seven Indiana counties included in the development of *C* values for the native flora of the Chicago region (Swink & Wilhelm 1994) and *C* values have recently been independently created for the State of Indiana (Rothrock 2004), there are two sets of *C* values available for the FQA of Silver Lake. Thus, *C* values from both sources were recorded for the native aquatic vascular taxa collected during the surveys of Silver Lake. These taxa have been assigned to conservatism groups based on their corresponding *C* values and their respective source (Table 1). Each conservatism group is defined by a specific range of *C* values (see Table 1). Nonnative and native taxa currently without *C* values have been assigned to group A (Table 1) and were excluded from the calculations of the FQA metrics described below.

Swink & Wilhelm's (1994) FQA of an area requires a mean coefficient of conservatism (C_{mean}) value, which is calculated as follows:

$$C_{mean} = \sum C/N$$

where ΣC is the sum of all *C* values of the native aquatic vascular macrophytes recorded from the lake and *N* is the total number of taxa having *C* values. The C_{mean} is used to calculate *I*:

$$I = C_{mean} * \sqrt{N}$$

where $\sqrt{N}$ is the square root of the total num-

Table 1.—Conservatism groups (modified from Alix & Scribailo 1998) for taxa collected from Silver Lake based on two sets of *C* values: IN = Indiana (Rothrock 2004); CR = Chicago region (Swink & Wilhelm 1994). Groups are: A (native and non-native taxa without assigned *C* values and taxa with a *C* value of 0 or 1); B (taxa with a *C* value of 2 or 3); C (taxa with a *C* value of 4, 5, or 6); D (taxa with a *C* value of 7 or 8); E (taxa with a *C* value of 9 or 10). Each group indicates a different level of habitat fidelity: N = not yet determined; EL = extremely low; L = low; M = moderate; H = high; EH = extremely high.

Group			*C* value		Fidelity	
IN	CR	Taxon	IN	CR	IN	CR
A	A	*Chara braunii*	N	N	N	N
A	A	*Chara globularis*	N	N	N	N
A	A	*Chara foliolosa*	N	N	N	N
A	A	*Myriophyllum spicatum*	N	N	N	N
A	A	*Nitella flexilis*	N	N	N	N
A	A	*Riccia fluitans*	N	N	N	N
A	A	*Ricciocarpus natans*	N	N	N	N
A	C	*Ceratophyllum demersum*	1	5	EL	M
A	A	*Typha latifolia*	1	1	EL	EL
B	C	*Elodea canadensis*	3	5	L	M
B	D	*Juncus effusus*	3	7	L	H
B	C	*Lemna minor*	3	5	L	M
B	C	*Lycopus americanus*	3	5	L	M
B	D	*Persicaria hydropiperoides*	3	7	L	H
B	C	*Sagittaria latifolia*	3	4	L	M
B	C	*Stuckenia pectinata*	3	5	L	M
C	E	*Brasenia schreberi*	4	10	M	EH
C	C	*Carex comosa*	6	5	M	M
C	C	*Cephalanthus occidentalis*	5	5	M	M
C	D	*Heteranthera dubia*	4	8	M	H
C	D	*Lemna trisulca*	6	7	M	H
C	C	*Najas flexilis*	5	6	M	M
C	D	*Nuphar advena*	6	7	M	H
C	D	*Nymphaea odorata* subsp. *tuberosa*	6	7	M	H
C	E	*Peltandra virginica*	6	10	M	EH
C	C	*Persicaria amphibia*	4	4	M	M
C	E	*Pontederia cordata*	5	10	M	EH
C	D	*Potamogeton foliosus* subsp. *foliosus*	4	7	M	H
C	D	*Potamogeton pusillus* subsp. *tenuissimus*	4	7	M	H
C	E	*Saururus cernuus*	4	9	M	EH
C	D	*Spirodela polyrrhiza*	5	7	M	H
C	E	*Utricularia gibba*	4	10	M	EH
C	E	*Utricularia macrorhiza*	5	9	M	EH
C	D	*Wolffia brasiliensis*	6	7	M	H
C	D	*Wolffia columbiana*	5	7	M	H
C	E	*Zannichellia palustris*	6	10	M	EH
D	E	*Eleocharis palustris*	8	10	H	EH
D	E	*Sparganium emersum*	8	10	H	EH
D	D	*Vallisneria americana*	7	7	H	H
E	E	*Ceratophyllum echinatum*	10	10	EH	EH
E	E	*Myriophyllum verticillatum*	10	10	EH	EH
E	E	*Potamogeton amplifolius*	10	10	EH	EH

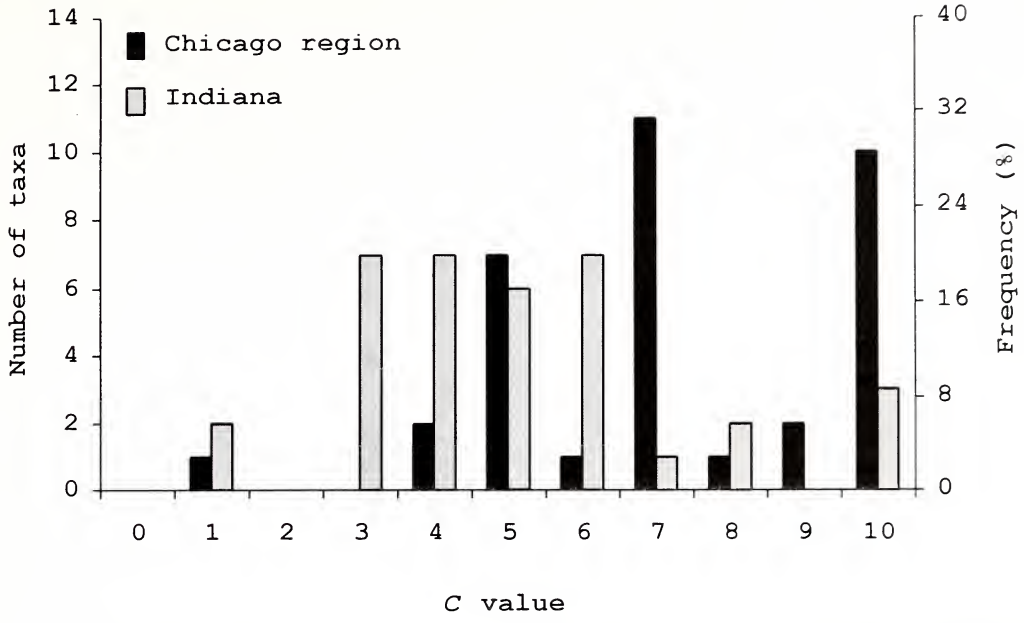


Figure 5.—Frequency distributions of aquatic vascular plant taxa recorded from Silver Lake grouped by *C* value. Frequencies represent the percentage of taxa with the same *C* value.

ber of taxa having *C* values and serves as an area-based standardization for species richness. These metrics were calculated twice for Silver Lake, first by using the Indiana set of *C* values and second by using the set from the Chicago region. Although sites surveyed at Silver Lake were equal in inventory area, the lake proper was defined as the inventory unit. Therefore, *I* and C_{mean} values were not calculated for individual survey areas, though the utility of these metrics when applied to individual samples has been demonstrated (Swink & Wilhelm 1994; Herman et al. 1996; Taft et al. 1997). Unlike other metrics, FQA does not rely on a suite of indicator species or the labor intensive, and often time-consuming, measurements of abundance (Swink & Wilhelm 1994).

The frequency distributions and median *C* values from each set of *C* values (i.e., Indiana and the Chicago region) recorded for the taxa documented during the study were compared by Mann-Whitney *U* tests utilizing normal approximation (Zarr 1974). The nonparametric two-sample tests were conducted because these data do not meet the assumptions of normality required for the application of analogous parametric statistical tests. *P* values less than 0.05 are considered significant.

Systematics.—Taxonomy and nomenclature of vascular aquatic macrophytes follow familial treatments of the Flora of North America Editorial Committee (1997, 2000, 2002a, 2002b, 2005) with the following exceptions: Haloragaceae (Aiken 1981), Lamiaceae and Rubiaceae (Gleason & Cronquist 1991), and Lentibulariaceae (Taylor 1989). Taxonomic treatment of the Characeae follows Daily (1953) with nomenclatural revisions where necessary, and that of the Ricciaceae follows Mayfield et al. (1983). Abbreviations for nomenclatural authorities follow the rules recommended by Brummitt & Powell (1992) and are from the International Plant Names Database (2004).

Voucher specimens of all taxa reported herein have been deposited in the Aquatic Plant Herbarium of Purdue University North Central (indicated here as PUNC). Duplicate specimens of some taxa (see Appendix) have been prepared and sent to the Friesner Herbarium at Butler University (BUT).

Physical and chemical parameters.—Field and lab analyses of subsurface water samples from 15 locations within Silver Lake (Fig. 5) were conducted on 15 July 2004. Water temperature, pH, specific conductance, and salinity were measured *in situ*. Water temper-

ature and pH were measured using an IQ Model 150 multi-parameter meter with a stainless steel ion-selective field effect transistor (ISFET) probe. Specific conductance and salinity were measured using a YSI Model 85 handheld meter. Water samples for the analysis of total alkalinity were collected from 10 of the 15 locations using a polycarbonate vertical point water sampler. Total alkalinity was determined *ex situ* using the Gran titration method (Wetzel & Likens 1991). Water samples were stored in 125 ml Nalgene® bottles and kept on ice at ≈ 4 °C in a cooler for no longer than 2 h prior to analysis. Water depth was determined at each of the 15 locations using a 3.0 m braided polyester line weighted on one end and calibrated in 0.1 m increments.

RESULTS

The inventory of aquatic macrophytes of Silver Lake documented 42 taxa, including three (7.1%) state-listed species (*Ceratophyllum echinatum*, *Myriophyllum verticillatum*, and *Zannichellia palustris*), one (2.4%) exotic (*Myriophyllum spicatum*), four (9.5%) charophytes (*Chara braunii*, *Chara foliolosa*, *Chara globularis*, and *Nitella flexilis*), and two (4.8%) liverworts (*Riccia fluitans* and *Ricciocarpus natans*). These taxa represent 32 genera from 23 families. Vascular plant families having the greatest richness were the Lemnaceae and Potamogetonaceae with five and four taxa, respectively. Families represented by a single species are the Alismataceae, Araceae, Cabombaceae, Juncaceae, Lamiaceae, Najadaceae, Rubiaceae, Saururaceae, Sparganiaceae, Typhaceae, and Zannichelliaceae.

Three taxa, *Potamogeton foliosus* subsp. *foliosus*, *Myriophyllum verticillatum*, and *Nuphar advena*, occurred at ≥50% of the survey areas; the latter taxon occurring at >75% of the areas. *Brasenia schreberi*, *Vallisneria americana*, *Chara braunii*, *Ricciocarpus natans*, *Ceratophyllum echinatum*, and *Zannichellia palustris* were the rarest species, occurring at <5% of the survey sites, with the latter four species having been recorded from only a single site. Other species, particularly emergents, have been categorized as rare or infrequent in our annotated list (see Appendix). This rarity of emergent species has most likely resulted from a low sampling frequency of sites on or near the shoreline (i.e., only 10% of the total number of survey areas occurred 3 m or less from shore).

Application of the FQA methodology at Silver Lake resulted in a C_{mean} of 5.0 (N = 35) and an I of 29.6, using C values for Indiana (Rothrock 2004); however, a higher C_{mean} (7.2) and I (42.6) were calculated by using C values for the Chicago region (Swink & Wilhelm 1994). When Indiana C values were used in the calculation of I, less than 18% of the taxa had a C value ≥7, whereas over 82% of them had a C value ≤6 (Fig. 5). In contrast, over 68% of the taxa had a C value ≥7; and less than 32% of them had a C value ≤6 when the calculations were made using C values for the Chicago region (Fig. 5). Indiana C values of 0, 2, and 9 and the Chicago region C values of 0, 2, and 3 were not represented or included in calculations of their respective indexes. Results of the Mann-Whitney test revealed a significant difference between the frequency distributions of C values from the Chicago region and Indiana (Fig. 5; U = 939; Z = 3.88; two-tailed $P < 0.001$). In addition, Indiana C values represented by the Silver Lake taxa were at least one C value lower than those from the Chicago region (U = 809.5; Z = 2.35; one-tailed $P < 0.01$).

The analysis of Indiana conservatism groups indicated that 86% of the taxa exhibit extremely low-to-moderate fidelity (groups A, B, and C) and that only 14% of the taxa were represented by the higher fidelity Groups D and E (Fig. 6). In contrast, the Chicago region conservatism groups A and C were represented by 43%, whereas 57% of the taxa were represented by groups D and E (Fig. 6). Group B of the Chicago region was not represented by a single taxon. One apparent similarity between the two sets of conservatism groups is that approximately 56% of taxa exhibiting extremely low to moderate fidelity were classified as group C plants.

Chemical and physical water data are summarized in Table 2. Silver Lake appears to be circumneutral, having a mean pH of 6.93 and a mean total alkalinity of 55.2 mg $CaCO_3$/L (Table 2). These values indicate that Silver Lake has poor buffering characteristics and is likely kept circumneutral in part by the decomposition of floating remnant peat deposits. Measurements of specific conductivity are moderate and range from 233.5–354.1 µS/cm

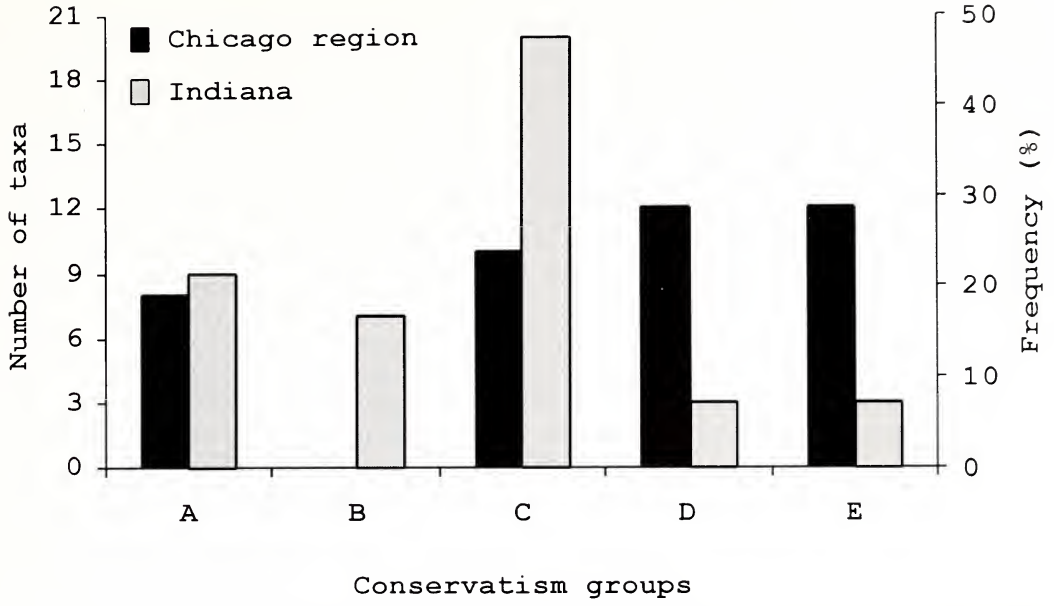


Figure 6.—Frequency distributions of aquatic macrophytes recorded from Silver Lake grouped by conservatism group. Frequencies represent the percentage of taxa within a group.

(Table 2). As expected of freshwater systems, salinity is low, having a mean value of 0.1 ppt and a statistical range of 0.1 ppt across survey areas (Table 2).

DISCUSSION

Successional history of Silver Lake.—If anecdotal accounts of the pre-burn flora of Silver Lake are correct, this area once represented an extremely unusual community of vegetation since an oak-history composition typically characterizes an upland forest community. It is possible that extensive ditching around the lake could have lowered the water table sufficiently to make the habitat very dry. This would also explain why the substrate caught fire so readily and burned down to the lake basin. However, a more plausible and typical successional history is that the lake was once a swamp, containing such species as swamp white oak (*Quercus bicolor*) and pin-oak (*Quercus palustris*), where sphagnum moss slowly invaded this area and eventually formed a peat substrate. The former two species are often found in bog habitats in the midwestern US, primarily as swamp remnants (Potzger 1934; Jones 1941). In general, the classic bog succession of the North, culminating in northern white cedar (*Thuja occidentalis*) or black spruce (*Picea mariana*) as dominants, is often not seen in northern Indiana and southern Michigan bogs where red maple (*Acer rubrum*), black ash (*Fraxinus nigra*) as well as tulip poplar (*Liriodendron tulipifera*) and oak are often common constituents (Transeau 1903; Cain 1928; Collins et al. 1979).

Since a substantial number of the original

Table 2.—Summary of physical and chemical parameters measured from sites within Silver Lake.

Parameter	*N*	Mean	Minimum	Maximum
Water depth (m)	15	0.6	0.2	0.9
Temperature (°C)	15	22.3	19.4	24.6
pH	15	6.93	6.43	7.26
Salinity (ppt)	15	0.1	0.1	0.2
Total alkalinity (mg $CaCO_3$/L)	10	55.2	44.1	66.4
Specific conductivity (μS/cm)	15	307.9	233.5	354.1

trees from the forest on Silver Lake are still present as submerged logs, we will continue our analysis of successional changes by obtaining wood samples for tree identification. It is also hoped that we may be able to locate undisturbed remnant peat deposits for coring and subsequent pollen stratigraphy analysis to document the longer term changes that led to the formation of a bog community.

Floristic quality of Silver Lake.—According to Swink & Wilhelm (1994), an assessment resulting in a C_{mean} value ≥ 3.5 and ≤ 4.5 or an I value ≥ 35 and ≤ 45 is indicative of an area having sufficient floristic quality to be at least of marginal natural area quality. Furthermore, areas having a C_{mean} or an I value exceeding the aforementioned values are floristically important from a statewide perspective, suggesting the presence of relatively high quality habitat(s) having natural area potential (Swink & Wilhelm 1994). The two sets of FQA metrics calculated for the Silver Lake flora provide somewhat contradictory results. Based on Swink & Wilhelm's (1994) suggestions, the C_{mean} value (5.0) calculated from Indiana C values (Rothrock 2004) indicates that Silver Lake is high in natural quality; however, the I value (29.7) indicates that Silver Lake possesses insufficient conservatism and richness. When the calculations were made using C values for the Chicago region (Swink & Wilhelm 1994), we observed a 42% increase in both the C_{mean} value (7.1) and I value (42.3), indicating that Silver Lake has high floristic quality and characteristics of a remnant natural area.

In this study, it appeared acceptable to use either source of C values because Porter County is in Indiana and it is also one of seven Indiana counties included in the Chicago region by Swink & Wilhelm (1994). However, the statistical comparison of the Indiana and Chicago region distributions of C values represented by the Silver Lake taxa revealed that they are significantly different. A similar result was obtained by Rothrock & Homoya (2005) when they compared the C values for the State of Indiana (Rothrock 2004) with those of the Chicago region (Swink & Wilhelm 1994) by examining the divergence of C values on a species by species basis and the mean divergence within specific conservatism cohorts. Rothrock & Homoya (2005) found that only 30% of taxa common to both Indiana and the Chicago region were assigned to the same cohorts. In addition, Rothrock & Homoya (2005) have shown that over 33% of Indiana taxa diverged from the C values of the Chicago region by 1–3 cohorts and that on average the Indiana C values were 1.2 cohorts lower than those of the Chicago region.

One question that arises from this study is which source of C values most accurately defines the floristic and natural area quality of Silver Lake? Based on our surveys and assessments of over 100 lakes and ponds in Indiana, a majority of which have been conducted in the Indiana counties of the Chicago region and have resulted in the documentation of many new localities for state-listed aquatic macrophytes (Scribailo & Alix 2002a), it appears that many C values assigned to aquatic vascular plants of the Chicago region (Swink & Wilhelm 1994) are inflated and that the Indiana C values (Rothrock 2004) are more appropriate. Nonetheless, we would certainly also question some of the Indiana C values assigned to aquatic vascular plants from Rothrock (2004) and will address this issue in a future paper.

Our overall assessment of Silver Lake indicates that it is low in natural area quality. This conclusion is based on additional factors admittedly not included in standard FQA methodology (Swink & Wilhelm 1994), such as the frequency of taxa, the history of the site, and the negative impacts of repeated drawdown events on the stability of habitat for a submerged flora. Having stated this, it is important to note that Silver Lake contains some floristically significant taxa, such as *Myriophyllum verticillatum* and *Ceratophyllum echinatum*.

Myriophyllum verticillatum (whorled watermilfoil), which is state-listed as rare by the Indiana Department of Natural Resources (Division of Nature Preserves 2004), forms extensive beds in deeper portions of Silver Lake. These beds encompass the largest flowering population of *M. verticillatum* we have seen in the state. The only other significant flowering population of *M. verticillatum* we have observed in Indiana is located in the sloughs east of the gravel pit ponds at the Jasper-Pulaski Fish and Wildlife Area. It is of interest that *Myriophyllum spicatum* (Eurasian watermilfoil), which is the one of the most troublesome invasive submersed species in Indiana

lakes, and much of North America (Grace & Wetzel 1978; Nichols & Shaw 1986; Smith & Barko 1990;), is only found in a few small pockets of the lake and does not appear to be expanding its population size.

Myriophyllum verticillatum is typically found in circumneutral waters of relatively low alkalinity (Moyle 1945; Crow & Hellquist 1983; Nichols & Yandell 1995). Most lakes in Indiana are alkaline with a pH above 8; therefore, habitat of the type found at Silver Lake is rare in the state. Silver Lake is the only lake we have surveyed with a mean pH <7. This fact should also have some bearing on conservation decisions regardless of what the results of FQA indicate.

Ceratophyllum echinatum (prickly coontail) is a recent addition to the aquatic flora of Indiana (Scribailo & Alix 2002b) where it is state-listed as rare (Division of Nature Preserves 2004). The occurrence of this species at Silver Lake marks its fifth documented record for Porter County and its sixteenth for the state, which includes Lake, La Porte, St. Joseph, Steuben, and Warren counties (Scribailo & Alix 2002b). *Ceratophyllum echinatum* was found growing with *Ceratophyllum demersum*, one of the most common aquatic vascular plant species in Indiana (unpubl. data), at one inventory site located in the southwestern corner of the lake. Observations of *C. echinatum* and *C. demersum* in Indiana indicate that the latter species is a common associate of the former (Scribailo & Alix 2002b). In Indiana, *C. echinatum* has been primarily found in shallow, mesotrophic to eutrophic lakes, having muck-type bottoms and waters that are circumneutral to moderately alkaline in pH (Scribailo & Alix 2002b). In Silver Lake, *C. echinatum* is restricted to a portion of the lake where the influx of sediments from surface runoff creates high turbidity.

The presence of some floristically significant taxa, a C_{mean} value >4.5, and the low relative frequency of *Myriophyllum spicatum* indicate considerable potential for maintenance and enhancement of floristic quality in Silver Lake, assuming that the appropriate management practices are adopted and enforced. A primary management concern impacting the floristic quality and ecological health of Silver Lake is that of hydrology. Over the past three years, we have observed drawdowns of up to 0.7 m in Silver Lake's water levels with the most significant and drastic drawdown occurring in the growing season of 2005. This drawdown transformed approximately 50% of the submersed habitat into mud flats. Muskrat, which normally maintain open channels through the spatterdock, creating habitat for the state-listed species recorded here, could not forage under these conditions. This drawdown also allowed spatterdock to colonize these areas that formerly were habitat for submerged plants.

Silver Lake is too shallow to withstand prolonged periods of drawdown, which may eventually eradicate floristically significant aquatic plant taxa as well as most other submerged species. Options to increase the extent of deep-water habitat in Siver Lake and to ameliorate the impacts of drawdown, such as establishing a state-mandated lake level and dredging, should be considered to reduce further degradation of the lake.

Problems associated with the use of FQA metrics for lakes.—Although we have used the FQA methodology (Swink & Wilhelm 1994) for our analysis of Silver Lake, we perceive several fundamental problems with the use and adequacy of this method as a diagnostic tool in evaluating lake ecosystems. The first of these shortcomings is that assessments of floristic quality of lakes have almost invariably failed to include characean algae (charophytes). The ecological attributes of charophytes make them important contributors to the stability of floristic quality and overall ecosystem health of lakes. These macrophytic algae often form extensive meadows in the littoral zone of lakes and ponds and are an integral component in both the structure and function of aquatic ecosystems (see Hutchinson 1975; Jeppesen et al. 1998; Coops 2002). The significance of charophytes in the conservation, management, and restoration of water bodies has recently been a source of considerable discussion (Van den Berg et al. 1998; Coops 2002). As we have suggested in the past (Alix & Scribailo 1998), *C* values must be assigned to charophytes at the species level, and in some cases the subspecific ranks, if the goal is to improve the effectiveness of the FQA metrics in evaluating lake ecosystems. It should be noted that there are at least 25 charophyte species in the Great Lakes region alone (unpubl. data) of an estimated 400 species world-wide (Moore 1986), thus not to

use charophytes in the assessment of floristic quality of lakes is akin to leaving out the Cyperaceae in the floristic quality assessment of wetlands.

A step in the right direction was taken by Nichols (1999), who assigned a single *C* value to the genera *Chara* and *Nitella* during his development and calibration of FQA metrics for macrophyte communities in Wisconsin lakes. However, the grouping of all charophyte species by genus and assigning a single *C* value to each group results in a loss of information and may not provide an accurate evaluation of the floristic quality of a given site.

We recognize that the morphological characters of charophyte species often vary markedly, which can make the identification of these species difficult even if one is fairly acquainted with the taxonomy of this group. Much of the current difficulty with charophyte identification was engendered by Wood's (1965) treatment of the Characeae in which numerous taxa were lumped under individual species and assigned subspecific ranks. Many of these taxa are now considered "good species" (e.g., see Proctor 1975). Unfortunately, no complete revisionary manual for the charophytes, of North America in particular, has been published since Wood (1965). Despite this problem, it is still important that efforts be made to include them in the FQA since many charophyte species show varying levels of fidelity to different aquatic habitats (Mann et. al. 1999). We are currently evaluating appropriate *C* values for the charophytes of Indiana. These values will be included in a paper that will present our assessment of *C* values for submersed and floating aquatic plants of Indiana.

A second problem with the use of the FQA for lakes, as Nichols (1999) suggested, is that lakes differ in a number of significant ways from terrestrial and wetland areas. Thus, the FQA metrics need to be calibrated to reflect these differences. For example, Nichols' (1999) examination of data on plant communities in Wisconsin lakes revealed that 95% of 554 natural lakes and man-made flowages had a C_{mean} value ≥ 3.5 and that only 8% had an *I* value ≥ 35. Furthermore, the average *C* value of 128 aquatic taxa from his study was 7.4, and 90% of these taxa had a *C* value ≥ 5. Since *I* values indicate that these lakes possess marginal floristic quality despite having a substantial number of highly conservative taxa, it is evident that the standard threshold values of the FQA metrics proposed by Swink & Wilhelm (1994) do not adequately serve as a gauge of the floristic quality of the Wisconsin lake plant communities. Nichols (1999) indicated that the low *I* values are often a product of low species richness, and that in contrast to terrestrial and wetland habitats, lakes are defined areas having a limited selection of species. Therefore, floristic surveys of terrestrial and wetland habitats may often result in the documentation of a far greater number of taxa and a higher *I* value than those of lakes since a higher *N* will increase *I* independently of any other factor.

To attempt to produce metrics useful in distinguishing floristic quality and natural area value among Wisconsin lakes and flowages, Nichols (1999) calibrated their values on both a statewide and an ecoregional-lake type scale. This type of calibration allowed for the comparison of the FQA metrics of lakes on two scales and avoided the reliance on inappropriate threshold values. Nichols (1999) has shown that when the metrics are calibrated, the median C_{mean} values of Wisconsin's four ecoregional-lake type groups only dropped by approximately half a unit (0.5) between each group from the highest value (6.7) to the lowest one (5.0). This is counter-intuitive given considerable diversity in the ecoregions of Wisconsin (Omernik et. al. 2000) and reinforces the argument for calibrating the FQA metrics specifically for lakes.

Nichols (1999) also found that lakes in Wisconsin having both the highest and lowest *I* values are man-made and suggested that the floristic quality of a lake is more likely related to its water quality than to its mode of origin. This suggests that attempting to assign *C* values to aquatic macrophytes based upon their fidelity to pre-settlement conditions may be problematic and indicates that man-made lakes may have a C_{mean} value equal to or greater than that of natural lakes.

An additional problem with the FQA of lakes is that non-native aquatic plant taxa are excluded from the calculation of the metrics used in this methodology. Typically, non-native taxa, whether they are terrestrial or aquatic, are not formally assigned *C* values and thus are not included in Swink & Wilhelm's (1994) FQA. It is widely recognized that non-native

taxa have a negative impact on the floristic and natural area quality of their respective habitats because they are often weedy and aggressive, displacing native species. Given this is the case; we believe that some effort should be made to provide additional information that might indicate the extent of this negative impact on habitat quality.

A proposed solution to this problem is to calculate the FQA metrics twice, first by including only native taxa and second by including native and non-native taxa, with the latter having been assigned a default value of $C = 0$ (Taft et al. 1997; Wilhelm & Masters 1999; Rothrock 2004; Rothrock & Homoya 2005) and then compare the results. This alternative approach, as recently suggested by Rothrock & Homoya (2005), "allows one to assess both the natural value of a site and the somewhat separate question of how much impact adventive species are having on the site." Examples, which use a default value of $C = 0$ for non-native taxa, can be found in Rothrock & Homoya (2005, Appendix), who provide FQA metrics, representing a diversity of sampling efforts and inventory units, for a variety of community types in Indiana.

Although the utility of this approach appears evident, we contend that this method will prove to be largely uninformative in assessing the impact of non-native taxa on lake ecosystems. The primary reason for this is that lakes tend to contain far fewer non-native taxa than their typical terrestrial counterparts, and yet the impacts of these taxa on the quality and natural value of lake habitats are often far more extensive and severe. For example, we have not encountered a water body with greater than four non-native submersed plant species during our inventories of Indiana lakes and ponds. The four species most commonly encountered are *Myriophyllum spicatum*, *Egeria densa*, *Najas minor*, and *Potamogeton crispus* (unpubl. data). A similar situation exists in Wisconsin where Nichols (1999) reported less than six non-native aquatic plant taxa for the entire Wisconsin lake flora. In contrast, the mean number of non-native taxa from 29 examples of terrestrial and wetland habitats provided by Rothrock & Homoya (2005, Appendix) is 24 and the range is from 0–148. Sixty-five percent of these habitats have five or more non-native species, and at least 30 non-native species are generally present at a given site before a reduction in I of 5.0 or more units is observed.

Although not explicitly stated by Alix & Scribailo (1998), the low number of non-native submersed aquatic taxa in the flora of Indiana and their insignificant impact on I was the basis of their support and promotion of the assignment of negative C values to non-native plant taxa, a practice used in an earlier index to evaluate natural areas (Swink & Wilhelm 1979). It is obvious that the use of negative C values for non-native aquatic taxa will reduce the values of ΣC, C_{mean}, and I further than the use of a default value of $C = 0$ for these taxa. What is not as obvious is whether the reduction in the I value is indicative of the level of negative change occurring in the floristic or natural area quality of the habitat. Using Swink & Wilhelm's (1994) defined threshold I values of 20, 35, and 50, which represent areas that are not floristically significant, regionally significant, and rare and highly significant, respectively, it is apparent that a reduction in I of 15 or more units would suggest substantial degradation. We have already shown that such a decrease would be extremely unusual for lakes and would possibly constitute the entire range of variation of I across all lakes.

It is apparent that neither treatment of non-native taxa (i.e., the assignment of a default value of $C = 0$ or the assignment of negative C values) in the FQA is likely to reveal the extent of the current negative impact of these taxa on a given habitat nor is it likely to indicate any change in their invasive spread. Ultimately, this means that without a change in the composition or number of taxa the floristic quality metrics will not change whether the population of a non-native species consists of a few individuals or thousands. Conventional wisdom holds that the spread of a non-native taxon will impact the FQA metrics indirectly by reducing the C_{mean} of a given area because of the loss or replacement of conservative taxa (Swink & Wilhelm 1994). The problem with this idea is that a lake could undergo substantial degradation in quality with the spread of a non-native taxon before it had significant effect on the values of the floristic quality metrics. Non-native aquatic species, such as *Egeria densa*, *Eichhornia crassipes*, *Myriophyllum spicatum*, and *Salvinia molesta* often occur in large monospecific stands, dominat-

ing lake plant communities and out-competing native plant taxa for light and space. Nonetheless, highly conservative taxa are generally still present in the aquatic flora, though they may have suffered a large reduction in numbers of individuals. Therefore, it is apparent that if non-native taxa are to be included in the FQA, with the goal of determining the impact of these taxa on habitat quality, that some quantitative or semi-quantitative description of their frequency should be incorporated into the FQA methodology.

Since their inception, FQA metrics have been derived without considering species abundance (i.e., density or cover) or frequency. It has been argued that these parameters are influenced by seasonal and yearly fluctuations and thus are considered to be irrelevant when assessing the qualitative value of a site (Swink & Wilhelm 1994). Although this is arguably true for native taxa, this is not the case for non-natives where documenting their increase in frequency or abundance is critical in a management context.

It is important to recognize that the choice of parameters used in describing the extent of the non-native component of plant community structure determines the overall quality of impact assessment. Frequency, based on presence-absence techniques, is less dramatically influenced by seasonal fluctuations in lake plant community structure than measurements of abundance, such as density or cover (Swindale & Curtis 1957; Nichols 1984; Nichols 1997; Madsen 1999). Although frequency varies with quadrat size or sample area (Greig-Smith 1964; Mueller-Dombois & Ellenberg 1974; Titus 1993) and is somewhat dependent on the density and distribution of taxa (Greig-Smith 1964; Mueller-Dombois & Ellenberg 1974), it is also the easiest quantitative measure to determine (Greig-Smith 1964; Mueller-Dombois & Ellenberg 1974; Nichols 1984; Madsen 1999). The most preferable measure of frequency for the assessment of the impact of non-native taxa on floristic quality is relative frequency because it takes into account the frequency of non-native taxa relative to all taxa at a given site.

Utilizing relative frequency, we propose a more informative method for assessing the impact of non-native taxa (rather than assigning them a default C value of 0 or -10). The first step in this process is to develop a separate floristic quality index value with non-native impact (I_{nni}) by calculating a specific impact term for non-native taxa (T):

$$T = \sum R_{nn} * I$$

where ΣR_{nn} is the sum frequency of non-native aquatic taxa divided by the sum frequency of all aquatic taxa for the inventory unit (i.e., sum relative frequency of non-native taxa) and I is the floristic quality index calculated without the inclusion of non-native taxa (Swink & Wilhelm 1994). The value of T can then be used to calculate I_{nni}:

$$I_{nni} = I - T$$

where I is the floristic quality index (as described above). One advantage of proposing this method is that it does not involve altering the C_{mean} (by the addition of non-native taxa) or compromise the integrity of the original equation for I. The utility of this method becomes apparent when we consider changes in the value of I using default C values for *Myriophyllum spicatum* at Silver Lake versus changes in I as a response to a hypothetical increase in the relative frequency of this taxon as shown below.

When the default values of $C = 0$ and $C = -10$ are assigned to *Myriophyllum spicatum* and used in the standard FQA calculations of C_{mean} and I, the original metric values of 5.0 and 29.6 are reduced to 4.9 and 29.2 and 4.6 and 27.5, respectively, regardless of the relative frequency of this species at Silver Lake. However, the calculations of I_{nni} using the hypothetical increase in the relative frequency of *M. spicatum* from 0.016 to 0.08, and to 0.4, result in a reduction of I from 29.1 to 27.2 to 17.8. Therefore, proportionately increasing the relative frequency of *M. spicatum* decreases the value of I_{nni} by a percentage equivalent to the change in T. This provides an indirect measure of the spread of this species, which is interpreted to be indicative of a negative impact on the aquatic macrophyte community. Changes in the original I value can also be examined to concomitantly indicate if species richness or C_{mean} has been significantly altered as this species continues to increase in relative frequency.

It is important to note that an increase in the relative frequency of non-native taxa has a greater influence on I_{nni} values of floristically significant and high quality habitats than on

those that are of marginal quality. For example, a habitat with an I value of 60 and a sum relative frequency of non-native taxa of 0.30 will result in an I_{nni} value of 42, whereas a habitat having an I value of 30 and the same relative frequency of non-native taxa will result in an I_{nni} value of 21. Given that the encroachment and impact of non-native taxa is more likely to reduce the relative frequency of highly conservative taxa than that of generalists, it would seem appropriate that the corresponding influence on the value of I_{nni} should reflect this.

Although we suggest that the sum relative frequency of non-native taxa is appropriate for the calculation of T, it is appealing to substitute this parameter with a measure of percent cover, especially since many lake assessments often involve the rapid collection of coverage data on only non-native taxa. A good example of this is the simple GPS mapping of patches or large beds of non-native taxa in lakes, where percent cover of these taxa is typically calculated as a portion of the total surface area of the water body or littoral zone. As a cautionary note, estimates of percent cover will most likely result in much greater values of T than relative frequency since the former is not directly proportional to the number of native species present at a sample location. Thus, there is a decided loss of information and assessment value with the use of percent cover in the calculation of T and I_{nni}.

Throughout this discussion, we have outlined the utility of an impact term for non-native taxa and have provided a hypothetical example of its usage. Future studies that track the long-term spread of non-native taxa in lakes should provide more information on the effectiveness of this method in describing changes in floristic and natural area quality.

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APPENDIX

AQUATIC MACROPHYTES DOCUMENTED FROM SILVER LAKE, INDIANA

The annotated list of aquatic macrophytes collected from Silver Lake is arranged alphabetically within three phyla. A taxon preceded by an asterisk (*) is considered non-native, whereas a taxon preceded by a double asterisk (**) is relatively new to Indiana. Identification code, common name, habit type, frequency of occurrence (%), frequency category, and relative frequency (%) are included for each taxon, respectively. All specimens were collected and identified by the authors, and deposited in the Aquatic Plant Herbarium of Purdue University North central (PUNC). When collected and prepared, duplicates were sent to and deposited in the Friesner Herbarium at Butler University (BUT) in Indianapolis, Indiana. State-listed taxa have their corresponding state status noted in brackets (e.g., [Endangered], [Threatened], [Rare], or [Extirpated]) as assigned by the Indiana Department of Natural Resources (Division of Nature Preserves 2004).

PHYLUM CHLOROPHYTA (Green Algae)

Characeae C. Agardh (Muskgrass Family)

Chara braunii S.G. Gmel.: CHABRA; Braun's muskgrass; submersed; 2.4; rare; 0.3; BUT, PUNC.

Chara foliolosa Muhl. *ex* Willd.: CHAFOL; Leafy muskgrass; submersed; 14.3; infrequent; 1.9; BUT, PUNC.

Chara globularis Thuill.: CHAGLO; Fragile muskgrass; submersed; 21.4; occasional; 2.9; BUT, PUNC.

Nitella flexilis (L.) C. Agardh: NITFLE; Flexible stonewort; submersed; 14.3; infrequent; 1.9; BUT, PUNC.

PHYLUM HEPATOPHYTA (Liverworts)

Ricciaceae Dumort. (Liverwort Family)

Riccia fluitans L.: RIAFLU; Slender riccia; submersed and free-floating; 14.3; infrequent; 1.9; BUT, PUNC.

Ricciocarpus natans (L.) Corda: RIONAT; Purple-fringed riccia; free-floating; 2.4; rare; 0.3; BUT, PUNC.

PHYLUM MAGNOLIOPSIDA (Flowering Plants)

Alismataceae Vent. (Water-plantain Family)

Sagittaria latifolia Willd.: SAGLAT; Common arrowhead; emergent; 4.8; rare; 0.6; BUT, PUNC.

Araceae Juss. (Arum Family)

Peltandra virginica (L.) Schott: PELVIR; Arrow arum; emergent; 21.4; occasional; 2.9; BUT, PUNC.

Cabombaceae A. Rich. (Water-shield Family)

Brasenia schreberi J.F. Gmel.: BRASCH; Water-shield; floating-leaved; 4.8; rare; 0.6; BUT, PUNC.

Ceratophyllaceae Gray (Hornwort Family)

Ceratophyllum demersum L.: CERDEM; Common coontail; submersed and free-floating; 19.0; infrequent; 2.5; BUT, PUNC.

****Ceratophyllum echinatum*** A. Gray: CERECH; Prickly coontail; submersed and free-floating; 2.4; rare; 0.3; BUT, PUNC. [Rare]

Cyperaceae Juss. (Sedge Family)

Carex comosa Boott: CARCOM; Bristly sedge; emergent; 4.8; rare; 0.6; BUT, PUNC.

Eleocharis palustris L.: ELOPAL; Marsh spike-rush; emergent; 7.1; rare; 1.0; BUT, PUNC.

Haloragaceae R. Br. (Water-milfoil Family)

Myriophyllum spicatum L.: MYRSPI; Eurasian water-milfoil; submersed; 11.9; infrequent; 1.6; BUT, PUNC.

Myriophyllum verticillatum L.: MYRVER; Whorled water-milfoil; submersed; 52.4; common; 7.0; BUT, PUNC. [Rare]

Hydrocharitaceae Juss. (Frog-bit Family)

Elodea canadensis Michx.: ELOCAN; Canadian water-weed; submersed; 28.6; occasional; 3.8; BUT, PUNC.

Vallisneria americana Michx.: VALAME; Eel-grass; submersed; 4.8; rare; 0.6; PUNC.

Juncaceae Juss. (Rush Family)

Juncus effusus L.: JUNEFF; Soft rush; emergent; 4.8; rare; 0.6; BUT, PUNC.

Lamiaceae Lindl. (Mint Family)

Lycopus americanus Muhl.: LYCAME; American water-horehound; emergent; 4.8; rare; 0.6; PUNC.

Lemnaceae Gray (Duckweed Family)

Lemna minor L.: LEMMIO; Small duckweed; free-floating; 35.7; occasional; 4.8; BUT, PUNC.

Lemna trisulca L.: LEMTRI; Ivy-leaved duckweed; free-floating, 31.0; occasional; 4.1; BUT, PUNC.

Spirodela polyrrhiza (L.) Schleid.: SPIPOL; Giant duckweed; free-floating; 35.7; occasional; 4.8; BUT, PUNC.

Wolffia brasiliensis Wedd.: WOABRA; Brazilian water-meal; free-floating, 11.9; infrequent; 1.6; PUNC.

Wolffia columbiana H. Karst.: WOACOL; Columbian water-meal; free-floating, 11.9; infrequent; 1.6; PUNC.

Lentibulariaceae Rich. (Bladderwort Family)

Utricularia gibba L.: UTRGIB; Humped bladderwort; submersed and free-floating; 35.7; occasional; 4.8; BUT, PUNC.

Utricularia macrorhiza J. Le Conte: UTRMAC; Common bladderwort; submersed and free-floating; 14.3; infrequent; 1.9; BUT, PUNC.

Najadaceae Juss. (Water-nymph Family)

Najas flexilis (Willd.) Rostk. & Schmidt: NAJFLE; Slender water-nymph; submersed; 23.8; occasional; 3.2; BUT, PUNC.

Nymphaeaceae Salisb. (Water-lily Family)

Nuphar advena (Ait.) W.T. Ait.: NUPADV; Spatterdock; floating-leaved; 81.0; abundant and widespread throughout the lake; 10.8; BUT, PUNC.

Nymphaea odorata Ait. subsp. *tuberosa* (Paine) Wiersema & Hellq.: NYMODT; White water-lily; floating-leaved; 19.0; infrequent; 2.5; BUT, PUNC.

Polygonaceae Juss. (Buckwheat Family)

Persicaria amphibia (L.) Gray: PERAMP; Water smartweed; emergent; 11.9; infrequent; 1.6; PUNC.

Persicaria hydropiperoides (Michx.) Small: PERHYO; False water-pepper; emergent; 21.4; occasional; 2.9; BUT, PUNC.

Pontederiaceae Kunth (Pickerel-weed Family)

Heteranthera dubia (Jacq.) Small: HETDUB; Water star-grass; submersed; 7.1; rare; 1.0; PUNC.

Pontederia cordata L.: PONCOR; Pickerel-weed; emergent; 4.8; rare; 0.6; PUNC.

Potamogetonaceae Dumort. (Pondweed Family)

Potamogeton amplifolius Tuckerm.: POTAMP; Broad-leaved pondweed; submersed; 7.1; rare; 1.0; BUT, PUNC.

Potamogeton foliosus Raf. subsp. *foliosus*: POTFOF; Leafy pondweed; submersed; 50.0; common; 6.7; BUT, PUNC.

Potamogeton pusillus L. subsp. *tenuissimus* (Mert. & Koch) R.R. Haynes & Hellq.: POTPUT; Small pondweed; submersed; 21.4; occasional; 2.9; BUT, PUNC.

Stuckenia pectinata (L.) Börner: STUPEC; Sago pondweed; submersed; 23.8; occasional; 3.2; BUT, PUNC.

Rubiaceae Juss. (Madder Family)

Cephalanthus occidentalis L.: CEPOCC; Buttonbush; emergent; 11.9; infrequent; 1.6; BUT, PUNC.

Saururaceae E. Mey. (Lizard's-tail Family)

Saururus cernuus L.: SAUCER; Lizard's-tail; emergent; 4.8; rare; 0.6; PUNC.

Sparganiaceae Rudolphi (Bur-reed Family)

Sparganium emersum Rehmann: SPAEME; Narrow-leaved bur-reed; emergent; 11.9; infrequent; 1.6; BUT, PUNC.

Typhaceae Juss. (Cat-tail Family)

Typha latifolia L.: TYPLAT; Broad-leaved cat-tail; emergent; 28.6; occasional; 3.8; PUNC.

Zannichelliaceae Dumort. (Horned-pondweed Family)

Zannichellia palustris L.: ZANPAL; Horned-pondweed; submersed; 2.4; rare; 0.3; BUT, PUNC. [Rare]

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NEW RECORDS FOR THE ALIEN ORIENTAL WEATHERFISH, *MISGURNUS ANGUILLICAUDATUS*, IN THE LAKE MICHIGAN BASIN, INDIANA (CYPRINIFORMES: COBITIDAE)

Thomas P. Simon[1], **Greg Bright**[2], **Frank Veraldi**[3], **James R. Smith**[4], and **James R. Stahl**[4]: [1]U.S. Fish and Wildlife Service, 620 South Walker Street, Bloomington, Indiana 47401 USA; [2]Commonwealth Biomonitoring, 8061 Windham Drive, Indianapolis, Indiana 46214 USA; [3]U.S. Army Corps of Engineers, Chicago District, 100 N. Canal, Chicago, Illinois 60604 USA; [4]Indiana Department of Environmental Management, 100 N. Senate Avenue (MC65-40-2 Shadeland), Indianapolis, Indiana 46204-2251 USA

ABSTRACT. The oriental weatherfish, (*Misgurnus anguillicaudatus*), a native of southeast Asia, was collected from six locations in the Grand Calumet River, Lake Michigan basin, during the summer of 2005. This loach species was collected in the West Branch from the junction with the Indiana Harbor Canal west to Columbia Avenue, and from the East Branch upstream from the junction to the Kennedy Avenue bridge and throughout the Indiana Harbor Canal to 151st Street bridge. Females collected during mid-June had distended abdomens and were ripe with eggs. Female urogenital pore openings showed that eggs had been extruded and that the species was at the beginning of the spawning period. The discovery of reproducing populations of this alien species increases the total number of fish species known from Indiana to 213 species.

Keywords: Cobitidae, alien species, invasive exotic, nonindigenous species, ship ballast

The oriental weatherfish (*Misgurnus anguillicaudatus*) is a freshwater, subtropical species native to southeast Asia. The species was described from Chusan Island, China (Cantor 1842). The species is considered native to Cambodia (Tirant 1929), China (Walker & Yang 1999) including Hong Kong (Man & Hodgkiss 1981), India (Kapoor et al. 2002), Japan and Thailand (Masuda et al. 1984), Korea (Welcomme 1988), Laos (Kottelat 2001), Myanmar (Talwar & Jhingran 1991), the Russian Federation (Bogutskaya & Naseka 1996), Taiwan (Kottelat 1998), and Viet Nam (Kottelat 2001b).

This species proved successful in the aquarium fish trade and has been introduced widely into other countries where it has escaped and become naturalized (Welcomme 1988). The oriental weatherfish has been introduced and naturalized into Australia (Welcomme 1988), Germany (Freyhof & Korte 2005), Italy (Razzetti et al. 2001), Palau (Welcomme 1988), Phillipines (Welcomme 1988), Turkmenistan (Sal'nikov 1998), and the United States including Hawaii (Yamamoto 1992; Fuller et al. 1999).

During investigations of the Grand Calumet River during the summer of 2005, we found oriental weatherfish in the West Branch, East Branch, and the Indiana Harbor Canal. The purpose of this paper is to summarize current knowledge of this species in Indiana and the Lake Michigan drainage.

METHODS

Study area.—The Grand Calumet River comprises a small watershed located in northwestern Indiana (Fig. 1) where it encompasses about 17,500 ha. contained almost entirely within Lake County, Indiana. The Grand Calumet River is about 34 km long and has been designated an Area of Concern by the International Joint Commission. The Grand Calumet River and Indiana Harbor Canal occupy a low-relief area in the glacial bed of geological Lake Chicago. The general flow is sluggish and westward in the East Branch of the Grand Calumet River, east- or westward in the West Branch depending on Lake Michigan levels, and northward in the Indiana Harbor Canal, an artificial connection to Lake Michigan. Land-use disturbance in the area has

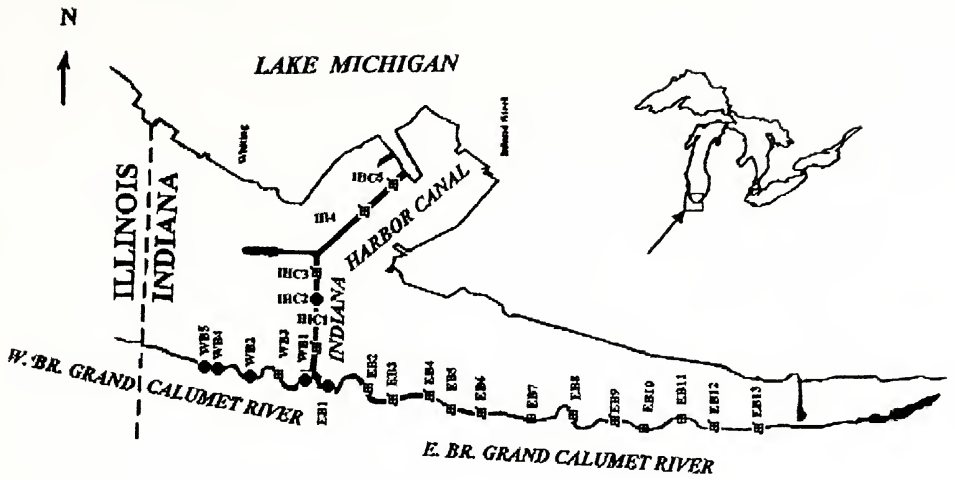

Figure 1.—Study area in the Grand Calumet River and Indiana Harbor Canal Area of Concern showing the 23 study sites (■). Black dots (●) show sites where oriental weatherfish were collected during 2002 and 2005.

been extensive with modification including ditching, channelization, flow modification, development of urban centers, and one of the most concentrated steel and petrochemical industrial complexes in the United States (U.S. EPA 1985).

Community collection and reach selection.—Fish communities were sampled along select reach segments based on previous water and sediment sampling stations above and below point-source discharges in the Grand Calumet River (Simon et al. 1989; Simon & Moy 2000; Simon et al. 2002). Nineteen reaches were evaluated between 13 June and 19 July 2005 to quantify fish assemblage presence in the Grand Calumet River and Indiana Harbor Canal (Fig. 1).

Twenty-three sites were surveyed to assess fish assemblage condition. The West Branch of the Grand Calumet River (WBGCR) had five reaches distributed between Columbia Avenue and the junction with the East Branch at 1) east of Columbia Avenue (WB5), 2) west of Indianapolis Boulevard (WB4), 3) east of Indianapolis Boulevard (WB3), 4) the East Chicago Sanitary District earthen channel (WB2), and 5) the mouth of the West Branch (WB1). The EBGCR had 13 reaches distributed between Broadway Avenue to the junction with the WBGCR. Collection reaches included 1) the mouth of the East Branch (EB1), 2) west of Kennedy Avenue (EB2), 3) east of Kennedy Avenue (EB3), 4) west of Cline Avenue (EB4), 5) east of Cline Avenue (EB5), 6) east of Clark Road (EB6), 7) east of Bonji (EB7), 8) west of Bridge Street (EB8), 9) east of Bridge Street (EB9), 10) east of Buchanan Street (EB10), 11) west of Buchanan Street (EB11), 12) east of the triple train trestles of USX (EB12), and 13) west of Broadway Avenue (EB13). We also sampled five locations in the Indiana Harbor Canal including 1) the area north of the junction (IHC1), 2) south of Columbus Drive (IHC2), 3) north of Chicago Avenue (IHC3), 4) north of 151st street (IHC4), and 5) south of Dickey Road (IHC5).

Fish species composition and relative abundance (catch-per-unit-of-effort or CPUE is the number of fish/minute of electrofishing effort) data were gathered by performing electrofishing surveys at each river reach using a model 6A Smith-Root boat-mounted electrofisher. Electrofishing surveys included systematic sampling of representative habitat within reaches, including the thalweg or deepest point in the cross sectional profile, usually for distances of 500 m for a minimum of 900 s. Captured fish were placed in an onboard holding tank until sampling was completed. Data recorded for each survey event included species identifications and weights, number of fish caught, examination for external disease and anomalies (DELTs), and sample and habitat conditions.

RESULTS AND DISCUSSION

The presence of reproducing populations of oriental weatherfish in the Grand Calumet River increases the number of naturalized fish species in Indiana to 213 species. We provide a brief history of the species world-wide introduction, occurrence in Indiana and Lake Michigan, and information on the species life history.

History of introductions.—The first reported introductions of *Misgurnus anguillicaudatus* were during the early 1900s until about 1924 from Asia to the Hawaiian Islands of Kauai, Maui and Oahu (Welcomme 1988). The species was introduced from northeast Asia to North America in 1939 for the aquarium trade. As a result of escapes from aquarium fish culture facilities, it became established in California, Idaho and some flood control channels in Michigan (Lake Huron)(Schultz 1960). The species has proven to be difficult to eliminate once established (Courtenay & Hensley 1980). In Trinidad Valley and rice terraces in Bontoc Province, Philippines, the species became established prior to 1937 in some waters where it forms the basis of a local fishery (Juliano et al. 1989). In 1984, the species was accidentally released by aquarists into Lake Burley Griffin, and in the Yarra and Owens rivers, Australia. It became established in the local streams around Canberra, Sydney, New South Wales, and in Victoria. The species was banned from importation to Australia as an aquarium fish due to its documented feral habits (McKay 1989). The only failed introduction of the species was in Mexico. The species had been cultured in the Chapingo Fish Hatchery, but has not been seen since the hatchery was closed (Contreras-B. & Escalante-C. 1984).

Distribution in Indiana and the Lake Michigan drainage.—Page & Laird (1993) and Laird & Page (1996) indicated that the species was probably established in the Chicago Ship Canal, Cook County, Illinois, by 1994 and in the North Shore Channel, Cook County, Illinois prior to 1987. The species was present in tributaries of Lake Michigan and in the Illinois River. Thus, occurrence in Indiana represents a natural range extension of the previously established population in northern Illinois. Nine species including rainbow smelt (*Osmerus mordax*), white perch (*Morone americana*), and round goby (*Apollonia melanostoma*), have crossed from Lake Michigan into the Illinois River system using the Chicago Sanitary and Ship Canal to expand their range from Lake Michigan to the Illinois River drainage. American eel (*Anguilla rostrata*), gizzard shad (*Dorosoma cepedianum*), goldfish (*Carassius auratus*), yellow bass (*Morone mississippiensis*), skipjack herring (*Also chrysochloris*), and oriental weatherfish have gone from the Illinois River into Lake Michigan.

Misgurnus anguillicaudatus was first collected in Indiana from the West Branch of the Grand Calumet River, Indiana, by Greg Bright and Mike Unger on 4 November 2002. Two specimens were collected using DC boat electrofishing near Hammond Sanitary District, Columbus Drive, Hammond. Earlier electrofishing during 1998 and 1999 by the U.S. Fish and Wildlife Service in the Grand Calumet River found no specimens. During 2002 a specimen was thought to have been observed in the vicinity near Hammond; however, it was not captured.

During June 2005, *Misgurnus anguillicaudatus* were collected from six locations in the Grand Calumet River, Lake Michigan drainage (Fig. 1). A total of 16 individuals was collected from the West Branch Grand Calumet River from all four sites sampled from the mouth of the West Branch at its junction with the Indiana Harbor Canal west to Columbia Avenue (WB1-4). A single specimen was collected from the East Branch Grand Calumet River from the mouth at its junction with the Indiana Harbor Canal upstream to the Kennedy Avenue bridge (EB1). Two specimens were found in the Indiana Harbor Canal upstream of the Columbus Avenue bridge (IHC3).

Habitat and life history.—The native habitat for the species includes mangrove swamps among stream and lake habitats (Kottelat 1998). Oriental weatherfish occur in rivers, lakes and ponds, as well as in swamps and rice fields (Berg 1948–1949). The species prefers muddy bottoms, where individuals hide in the muck and leaf litter with only their heads sticking out. In Hawaii, the species has also been found under mats of honohono (*Commelina diffusa*) and California grass (*Brachiara nuatica*). In our study area, the species was always found slightly buried in

the sediment near areas with woody debris or emergent wetland vegetation.

Oriental weatherfish are omnivorous (Lee et al. 1980), with their diet typically consisting of bottom dwelling animals, such as insect larvae, snails and worms (Sterba 1973). In its native habitat, the species' usual temperature range is from 10–25 °C; however, the species has been known from the Cal Sag Channel, Illinois at lower temperatures (Laird & Page 1996).

The species has a mean population doubling time of 1.4–4.4 yrs with mean female fecundity of 2000 eggs (Berg 1948–1949). We found females ripe with ova in mid-June. Females had urogenital pores that were distended and showed obvious evidence of eggs being extruded, suggesting that the spawning season had just begun.

In conclusion, species introductions, whether intentional or accidental, show that long-range spread of species can occur even after decades of relative inactivity. We do not know the ramifications of the presence of the oriental weatherfish from the Lake Michigan drainage; but based on the experience of others, it will be difficult to eradicate this species from the Lake Michigan nearshore and coastal wetlands. Since it is reproducing in the Grand Calumet River, it will probably not require too long before it will continue to spread elsewhere in the southern Lake Michigan drainage because of its short mean generation time and access through the Indiana Harbor Canal.

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SELECTION OF SONG PERCHES BY CERULEAN WARBLERS

Kelly Jones and Kamal Islam: Department of Biology, Ball State University, Muncie, Indiana 47306-0440 USA

ABSTRACT. Cerulean Warbler (*Dendroica cerulea*) populations have experienced serious declines in recent decades. Due to limited research on this species, a better understanding of habitat use and territory site selection at the regional level is needed. Within the Pleasant Run Unit of the Hoosier National Forest, Morgan-Monroe State Forest, and Yellowwood State Forest, a total of 43 Cerulean Warbler territories were mapped during 2004 and 2005. Within each territory, crown height, diameter at breast height (dbh), and species were recorded for trees used as song perches. Dbh, height measurements, and species were also recorded for trees within a 0.04 ha circle at the center of each territory. Cerulean Warbler song-perch trees were larger and taller than surrounding trees within territories. In addition, certain species were selected as song-perch trees, whereas others were avoided.

Keywords: Cerulean Warbler, *Dendroica cerulea,* song-perch trees, territory, Indiana

The Cerulean Warbler is a species of conservation concern; Breeding Bird Survey (BBS) data have demonstrated an annual population decline of 3.7% between 1966–1996 (Hamel 2000a). Only five other North American breeding bird species showed greater declines. Until recently, very little study had focused on this species (Hamel 2000b). Because of its dependence on large tracts of mature deciduous forest for successful breeding, much of its decline is likely due to extensive loss and fragmentation of forest tracts for agricultural use (Oliarnyk & Robertson 1996; Hamel 2000a).

Robbins et al. (1992) demonstrated that the Cerulean Warbler is a canopy-dwelling species. Among closely related species, this small bird spends most of its time higher in the canopy than other wood warblers (Hamel 2000a). Some studies have suggested that Cerulean Warblers use larger trees as song perches (Lynch 1981; Robbins et al. 1992; Hamel 2000a). However, given the substantial variation in habitat across its breeding range (differences in tree species composition, size of available trees, and forest tract size) and in behavioral variables (territory size and site fidelity), inquiry into song-perch tree characteristics on the regional level is essential (Hamel 2000a; Jones & Robertson 2001; Roth 2004). The purpose of this study was to determine if song-perch trees are larger and taller than surrounding trees within Cerulean Warbler territories in southern Indiana. Also, selection of specific tree species as song-perches was investigated.

Corresponding author: Kamal Islam: Department of Biology, Ball State University, Muncie, Indiana 47306-0440 USA; Phone: (765)-285-8847; fax #: (765)-285-8804; e-mail: kislam@bsu.edu

STUDY AREA

This study took place from 1 May to mid-August of 2004 and 2005 in the Pleasant Run unit of the Hoosier National Forest, Yellowwood State Forest, and Morgan-Monroe State Forest in Brown, Morgan, Lawrence, and Jackson counties, Indiana (Fig. 1). Historically, the Cerulean Warbler was one of the most abundant breeding warblers in the Ohio and Mississippi river valleys (Hamel 2000a). As a part of that area, forest blocks used in this study are among the largest and most unfragmented in southern Indiana.

METHODS

Bird surveys.—In each of 10 study sites, presence of male Cerulean Warblers was determined by walking seven transects within a 1.96 km^2 plot, with seven sampling points per transect, each point 200 m apart (Fig. 2). Transect point locations were recorded in Universal Transverse Mercators (UTMs) coordinates using Global Positioning System (GPS) receivers. To reduce edge influences, surveys were conducted > 50 m from roads. Bird surveys began 1 May, and were completed by 30

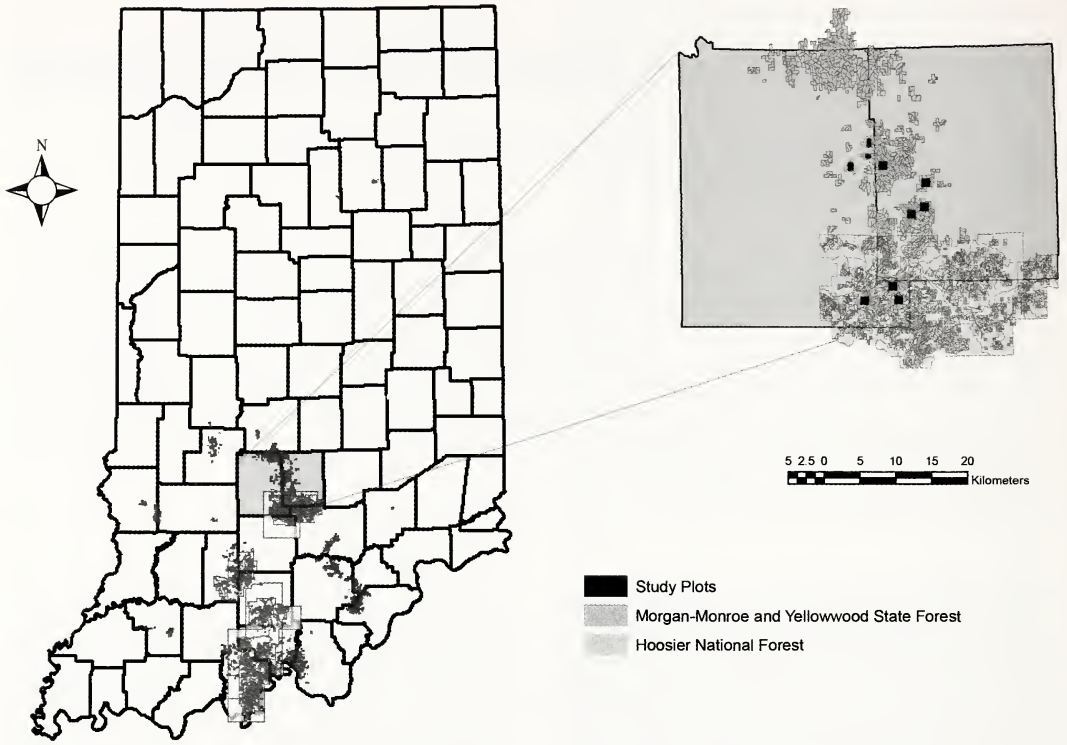


Figure 1.—County map of Indiana showing forest areas where Cerulean Warblers (*Dendroica cerulea*) were surveyed during 2004 and 2005.

May. Surveys were conducted between 0530–1030 h, excluding rainy days (presence of precipitation), when cessation or reduction of vocalizing may occur. At each survey point, 3 minutes of listening for Cerulean Warbler vocalizations commenced, followed by a 15 second playback of a conspecific male song in each of the cardinal directions to elicit a vocal response. This was followed by an additional three minutes of listening before moving to the next survey point (Falls 1981). The compass bearing and distance of detected males was estimated from the nearest transect point.

Territory mapping.—Most male Cerulean Warblers were relocated after initial detection during surveys because they maintain territories during the breeding season (Hamel 2000a). Once surveys provided the initial location of a male, territories were mapped by flagging a minimum of 5 trees in which males vocalized and perched. Singing from territory boundaries is the primary means of defending a territory and attracting potential mates (Hamel 2000a). For the purpose of this study, trees in which males vocalize are called song-perch trees. A territory is defined as the area within the perimeter of song-perch trees. UTMs of song-perch trees were recorded for ease in returning to the territory and calculating the territory center.

Vegetation measurement and analysis.—From early July to mid-August 2004 and 2005, vegetation was sampled within each of 43 territories, using the methods of James & Shugart (1970) outlined below. In territories, a 0.04 ha circular plot was marked at the approximate center of the territory. Diameter at breast height (dbh) and height were recorded for each tree in the plot with dbh $\geq$ 10 cm, and species for each tree with dbh $\geq$ 3 cm. A Nikon Laser 440™ compact rangefinder was used to determine tree heights. Dead trees were measured in the same manner as live trees. Dbh, height, and species were also recorded for all song-perch trees.

Dbh, height, and species were compared between trees from territory sample plots and song-perch trees. Results were calculated using all individual trees, as well as means calculated by territory. *t*-tests were used for com-

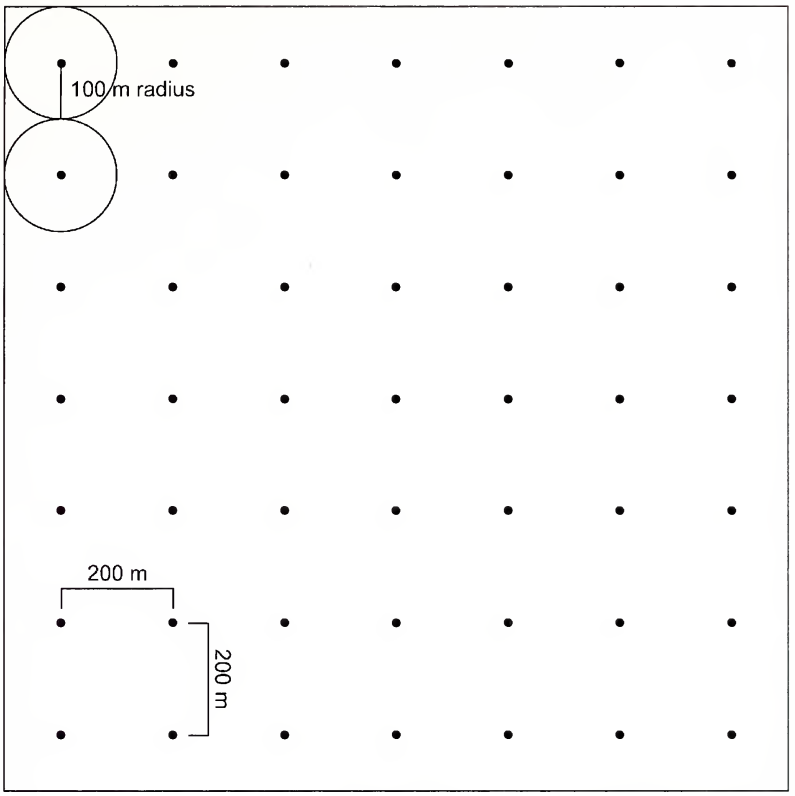


Figure 2.—The protocol utilized for Cerulean Warbler (*Dendroica cerulea*) surveys within ten study sites located in southern Indiana during the summers of 2004 and 2005. Seven transects, 200 m apart, were surveyed. Each transect contained seven points.

parison of individual trees, and paired *t*-tests were used for comparison of means. Chi-square analysis was used to compare tree species diversity between trees from sample plots and song-perch trees. Level of significance was set at $P = 0.05$.

RESULTS

Statistical analyses were computed using pooled data from 2004 and 2005. Mean values are reported as mean ± 1 SD. Mean number of Song-perch trees was 13.8 trees per territory (range 5–27). Cerulean Warblers used significantly larger ($\bar{x}$ = 43.0 ± 14.1 cm, n = 594, $P < 0.001$, t = 19.26) and taller ($\bar{x}$ = 26.9 ± 4.11 m, n = 591, $P < 0.001$, t = 18.10) trees for song perches than the average trees available within territories ($\bar{x}$ = 27.4 ± 15.6 cm, n = 751; $\bar{x}$ = 21.5 ± 6.04 m, n = 604, dbh and height, respectively) (Figs. 3, 4). Comparison of means by territory reflected the same pattern; perch trees were larger ($\bar{x}$ = 44.3 ± 6.3 cm, n = 43) and taller ($\bar{x}$ = 27.0 ± 2.2 m, n = 43) than surrounding trees ($\bar{x}$ = 27.7 ± 3.71 m, n = 43; $\bar{x}$ = 21.6 ± 2.0 m, n = 43, dbh and height, respectively) within territories (Figs. 5, 6).

Of the 39 tree species (including snags) present in territories, 12 were used more often than expected as song-perch trees (Table 1). Six species were used with less frequency than expected as song-perch trees (Table 1). Pawpaw (*Asimina triloba*), blue beech (*Carpinus caroliniana*), Ironwood (*Ostrya virginiana*), and grape (*Vitis* spp.), were never observed being used as song perches by Cerulean Warblers. Notably, 116 of 595 song perch trees in our study were white oak, and 105 were bitternut hickories. These two species made up 37% of all song perch trees in the study.

DISCUSSION

Within our study, only a handful (~2%) of song-perch trees within territories were < 15

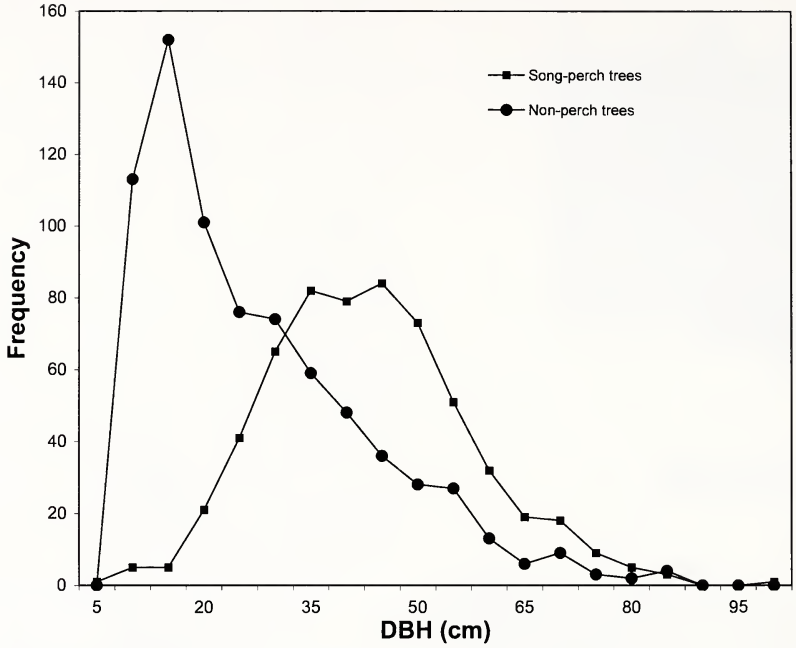


Figure 3.—Comparison of non-perch and perch tree dbh values within Cerulean Warbler (*Dendroica cerulea*) territories in southern Indiana during 2004 and 2005.

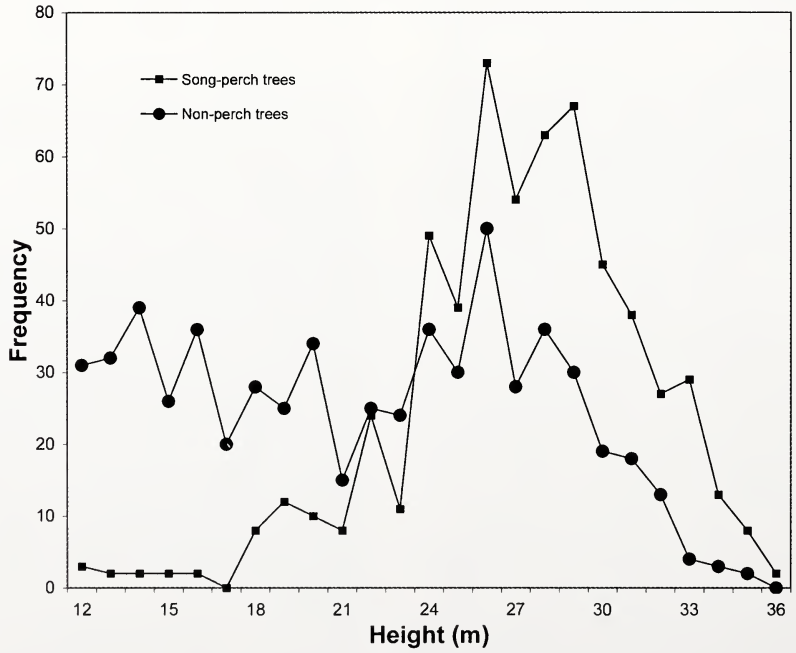
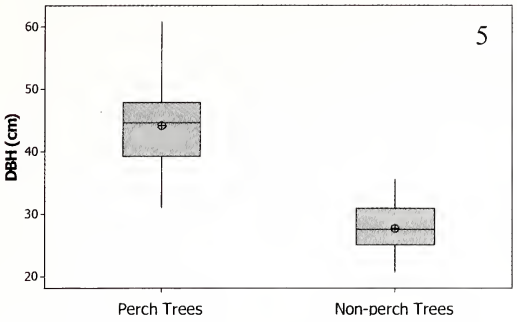
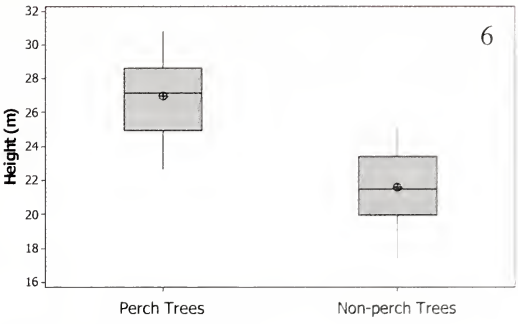


Figure 4.—Comparison of non-perch and perch tree height values within Cerulean Warbler (*Dendroica cerulea*) territories in southern Indiana during 2004 and 2005.





Figures 5, 6.—Comparison of non-perch and perch tree mean dbh and height by Cerulean Warbler (*Dendroica cerulea*) territory in southern Indiana during 2004 and 2005. 5. Means ± SD of Song-perch tree vs Non-perch tree dbh values; 6. Means ± SD of Song-perch tree vs Non-perch tree height values.

Figures 5, 6.—Continued.

m, and these were associated with canopy gaps or clearings. The vast majority of song-perch trees were relatively mature trees that were larger than those around them. Cerulean Warblers have long been known as canopy-dwelling birds (Robbins et al. 1992; Hamel 2000a). Robbins et al. (1992) noted that Cerulean Warblers were more often found in trees with larger dbh, and spent most of their time above the middle of the tree. In this study, song-perch trees had significantly larger dbh and height. Dbh and height are related, but there is much variation possible across individual trees, which gives value to comparison of both variables (Morey 1936).

Cerulean Warblers may also be selecting territories based on the presence of ideal song-perch trees. This will not always be apparent, as they will sing while foraging, preening, and in flight. However, when singing is the focus, they often choose and make return visits to exposed perches from which they sing (Hamel 2000a; KJ pers. obs.). Taller and larger trees may offer more high-quality song perches conducive to vocal projection. In a study of Golden-winged Warblers (*Vermivora chrysoptera*), song-perch trees were significantly larger than expected; and it was suggested that this song perch selection enhanced the birds' ability to display vocally and visually in mate attraction (Rossell 2001).

Much of the research on Cerulean Warbler habitat has focused largely on canopy structure, with less attention to tree species composition (Hamel 2000a, Jones and Robertson 2001, Weakland and Wood 2005). Oliarnyk (1996) and Hamel (2000b) reported no preference in Cerulean Warblers for tree species in nesting or foraging in Ontario and Tennessee populations, but song-perch tree preferences were not investigated. Among 13 species of foliage-gleaning birds in floodplain forests in southern Illinois, the Cerulean Warbler was the second most selective bird, closely following the Yellow-throated Warbler (*Dendroica dominica*), in tree species usage (Gabbe *et al.* 2002).

Gabbe *et al.* (2002) found that Cerulean Warblers in Illinois showed the strongest preference for shellbark hickory (*Carya laciniosa*) and bitternut hickory (*Carya cordiformis*). Tree species that were most strongly avoided were red maple (*Acer rubrum*) and blue beech. Shellbark hickory had such a low density overall in our study area, its usage by Cerulean Warblers cannot be compared to the study in southern Illinois. We also found that males selected bitternut hickories as song perches together with white oaks. Red maples were avoided, and blue beeches were never used for song perches. It appears that Cerulean Warblers are adaptable in their use of tree species across their breeding range, but where breeding areas have tree species in common, Cerulean Warblers appear to be fairly consistent in species selection.

Thirty percent of trees in territories were sugar maples, yet only 2.4% of song perch trees were of this species. On the other hand, white oaks and bitternut hickories represented only 5.1% and 3.7% of trees within territories, respectively, but 19.5% and 17.6% of song perch trees were of those species. The appearance of tree species selectivity in our study area may be due to a relationship be-

Table 1.—Thirty-eight tree species and snags identified within Cerulean Warbler (*Dendroica cerulea*) territories in southern Indiana during 2004 and 2005 in the Hoosier National Forest, Yellowwood State Forest, and Morgan-Monroe State Forest. An "X" indicates significantly higher than expected frequencies of tree species in Cerulean Warbler territory sample plots vs. song-perch trees. An asterisk indicates exclusive occurrence within the category.

Tree species	Scientific name	Territory sample plots	Song-perch trees
Red maple	*Acer rubrum*	X	
Sugar maple	*Acer saccharum*	X	
Downy Juneberry	*Amelanchier arborea*		
Hercules-club	*Aralia spinosa*		
Pawpaw	*Asimina triloba*	X*	
Blue beech	*Carpinus caroliniana*	X*	
Bitternut hickory	*Carya cordiformis*		X
Pignut hickory	*Carya glabra*		X
Shagbark hickory	*Carya ovata*		X
Eastern redbud	*Cercis canadensis*		
Flowering dogwood	*Cornus florida*	X	
American beech	*Fagus grandifolia*	X	
White ash	*Fraxinus americana*		
Green ash	*Fraxinus pennsylvanica*		X
Honey locust	*Gleditsia triacanthos*		
Black walnut	*Juglans nigra*		X
Eastern red cedar	*Juniperus virginiana*		
Spicebush	*Lindera benzoin*		
Tulip poplar	*Liriodendron tulipifera*		X
Black gum	*Nyssa sylvatica*	X	
Ironwood	*Ostrya virginiana*	X*	
Short-leaf pine	*Pinus echinata*		
Eastern white pine	*Pinus strobus*		
Sycamore	*Platanus occidentalis*		X
Big-toothed aspen	*Populus grandidentata*		
Black cherry	*Prunus serotina*		
White oak	*Quercus alba*		X
Chinquapin oak	*Quercus muehlenbergii*		X
Chestnut oak	*Quercus prinus*		X
Red oak	*Quercus rubra*		X
Black oak	*Quercus velutina*		X
Winged sumac	*Rhus copallina*		
Black locust	*Robinia pseudoacacia*		
Sassafrass	*Sassafrass albidum*		
Basswood	*Tilia americana*		
American elm	*Ulmus americana*		
Red elm	*Ulmus rubra*		
Grape species	*Vitis spp.*	X*	
snag	—	X	

tween species and size parameters. Sugar maples may not have been used in proportion to their abundance because many of them were not canopy trees, and being a canopy-dwelling species, Cerulean Warblers would not encounter them. However, even in territories where sugar maples made up the majority of canopy trees, some males avoided them completely during our observations. The only sugar maples recorded as song perch trees were concentrated in the territories of what appeared to be a few exceptional birds, most of which were unpaired. Further study addressing tree species importance, or at least importance of certain crown types associated with groups of species, would be worth pursuing.

This study demonstrated that in southern Indiana, Cerulean Warblers are utilizing the largest and tallest trees in their territories as song perches. These trees may offer individuals some advantage in territory defense and mate attraction, acoustically and/or visually. Cerulean Warblers in southern Indiana also used tree species disproportionately to their availability, just as they have been demonstrated to do in Illinois (Gabbe et al 2002). Exploration of more specific aspects of song perch selection (e.g. documentation of perch heights relative to tree heights, approximate girth of perch branches or twigs, foliage cover on perch) throughout the breeding range would be helpful in deepening our understanding of the specific habitat needs of Cerulean Warblers (Robbins et al. 1992).

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THE INDIANA MYOTIS (*MYOTIS SODALIS*) ON AN ANTHROPOGENIC LANDSCAPE: NEWPORT CHEMICAL DEPOT, VERMILLION COUNTY, INDIANA

Virgil Brack, Jr. and **John O. Whitaker, Jr.**: Department of Ecology and Organismal Biology, Indiana State University, Terre Haute, Indiana 47089 USA

ABSTRACT. Indiana myotis (*Myotis sodalis*) caught on Newport Chemical Depot, Vermillion County, Indiana used four primary and two alternate roosts, all within 6.3 km of one another, supporting the hypothesis of a fission-fusion social system. Roosting with larger numbers of individuals may be advantageous when thermoregulatory demands are high, while roosting with fewer individuals may help reduce parasitism and place individuals closer to foraging areas. Roosts and capture sites were scattered across a landscape dominated by agriculture and development, requiring that bats cross open lands and roads. One roost was on the edge of a 0.7 ha woodlot, adjacent to a four-lane divided highway. Movement across open spaces among habitat patches may readily occur because the species is adapted to a natural interspersion of open and wooded habitats. Loss or conservation of small or isolated wooded tracts within the core range may harm or benefit the species, and this effect may be proportionally greater than in portions of the range where wooded lands are plentiful.

Keywords: Anthropogenic, habitat, Indiana myotis, maternity roosts, *Myotis sodalis*, Newport Chemical Depot, radiotelemetry

The range of the Indiana myotis (*Myotis sodalis*) includes much of the eastern United States. The eastern portion of the range has more woodlands, larger woodland tracts, and less fragmentation, but fewer Indiana myotis than the western portion (Brack et al. 2002). Despite smaller wooded tracts, fragmented by agriculture and development, the Indiana myotis is more common in the core summer range of northern Missouri, southern Iowa, Illinois, northern Indiana, southern Michigan, and southwestern Ohio. Within the core range, only 24% of the land area in counties with records of reproductive Indiana myotis is forested (Gardner & Cook 2002). In the core range, reproductive individuals and maternity colonies have been found in small upland woodlots, many of which were grazed (cows, sheep, and pigs) and repeatedly high-graded for lumber, and in narrow riparian strips (USFWS 1999). Nevertheless, most studies of the Indiana myotis have focused on natural aspects of the habitats and ignored anthropogenic aspects of the landscape and habitat, and the implications that use of these areas has for management and conservation of the species. An exception is the studies at the Indianapolis airport (Sparks et al. 2005; Whitaker et al. 2004).

Kurta et al. (2002) showed that the Indiana myotis exhibits a social organization similar to the fission-fusion groups of many primates. Group size in primates is often limited by scarcity of food and availability of safe sleeping quarters; hamadryas baboons (*Papio hamadryas*) break into small groups to forage but congregate by hundreds on a few cliffs at night, while patas monkeys (*Erythrocebus patas*) disperse to sleep individually in trees relatively inaccessible to predators (Kummer 1979). Sussman (1979) cautioned that even among primates there is no simple relationship between social structure and phylogeny or ecology, so the fission-fusion social structure is best explained by the animals taking advantage of both group and independent living. For the Indiana myotis, the advantage of a maternity colony may be conservation of energy for thermoregulation; there may be a limited availability of thermally suitable roosts with adequate solar exposure (Carter & Feldhamer 2005; Humphrey et al. 1977; Kurta et al. 2002), and thermoregulatory demands of individuals may be less when in a group than

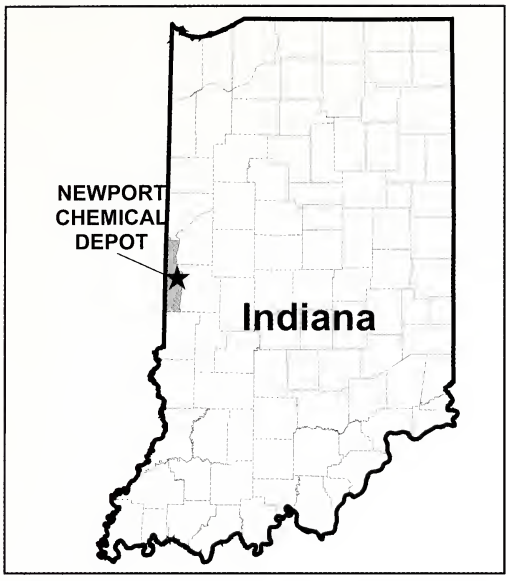

Figure 1.—Location of the Newport Chemical Depot in Vermillion County, Indiana.

when roosting singly (Tuttle 1975). In contrast, living in groups may increase risk of predation (Sparks et al. 2003), large colonies may be more susceptible to parasite infestations (Krutzsch 1955), and more bats are at risk when roost trees fall over. Typically, foraging for a dispersed or patchy resource, such as non-swarming insects, is by individuals or small groups; and use of roosts with fewer bats may place individuals closer to foraging areas.

The purpose of this study was to examine capture and roost sites of the Indiana myotis associated with Newport Chemical Depot, Vermillion County, Indiana, a landscape dominated by agriculture and development. Use of the area is examined in light of the fission-fusion theory of social organization and in terms of conservation of the species.

METHODS

Study areas.—This study was completed on Newport Chemical Depot, formerly the Newport Army Ammunition Plant, in Vermillion County, Indiana (Fig. 1), approximately 3 km southwest of the city of Newport and about 112 km west of Indianapolis. Newport Chemical Depot covers approximately 2873 ha, of which 770 ha are forested. Parts of the study extended outside the borders of Newport Chemical Depot. The study area is located at the interface of two natural regions: the Grand Prairie Section of the Grand Prairie Natural Region and the Entrenched Valley Section of the Central Till Plain Natural Region (Homoya et al. 1985). The Grand Prairie Section is characterized by dark, fertile, loamy soils, and as the name implies, included a great variety of natural prairie communities before European settlement. The Entrenched Valley Section, as the name implies, is identified by deeply entrenched valleys along major drainages. The Wabash River is the dominate drainage in this area. Except in specialized cliff and ravine communities, the hardwood forest communities are essentially the same as in most of the Tipton Till Plain Section, also a part of the Central Till Plain Natural Region. Upland forests, bottomland forests, and flatwoods are the major natural communities, although prairies, gravel-hill prairies, fens, marshes, savannas, cliffs, and seep springs are also present. The boundary between the two natural sections is not abrupt, but rather is a mosaic of prairie (typically converted to agriculture, although Newport Chemical Depot has 91 ha of restored prairie) and oak timber lands.

Bat captures and radiotelemetry.—Bats were captured with mist nets between 15 May and 15 August in 1987 (1 net night and 1 bat trap night), 1994 (12 sites; 12 net nights), 1997 (4 sites; 12 net nights), 1998 (10 sites; 18 net nights), and 2003 (4 sites; 18 net nights). The two areas where bats were caught in 1997 were netted in 1998, and all areas netted in 2003 were the same as those netted in 1997. Netting was completed with nets 6–20 m long and 2–6 m high. Netting was initiated at dusk and continued until between midnight and 0200 h.

Bats were removed from the net and identified to species. Mass, sex, age, right forearm length, and reproductive condition were recorded. In 1997, radiotransmitters were attached to two pregnant female Indiana myotis; and in 2003 two lactating females and a juvenile were fitted with transmitters (Holohil Systems, Ltd., Ontario, Canada and Titley Electronics, Pty., Ltd., Ballina, Australia). Transmitters weighed approximately 0.43 g and were attached dorsally between the scapulae using a non-toxic surgical adhesive (Skin-Bond® cement; Smith and Nephew, Inc., manufacturer). Bats were released at the

point of capture. Bats with transmitters were tracked to day roosts using Wildlife Materials Inc. (Carbondale, Illinois) model series TRX 1000 and 2000 receivers for 1–5 days following transmitter attachment. Roost tree locations, species, dbh, condition (live or dead), and amount of exfoliating bark were recorded. Each roost tree was watched at dusk 1–5 nights to count the number of bats exiting the roost. Roost emergence counts were completed in summer 1998 at the two roost trees found in 1997. Roosts used by multiple bats and/or on several visits were classified as primary roosts, while roosts used by a single bat were considered alternate roosts.

Net sites and roost trees were located with hand-held GPS units (Gamin model 12) during studies in 2003, but during prior studies sites were located by placing them on USGS 7.5 minute topographic maps while in the field. Roost height was considered as the level at which bats emerged for their evening exodus, and was visually estimated. Canopy closure at the roost was also estimated visually.

Habitat characterization.—To complete an analysis at the landscape scale, net sites where Indiana myotis were caught and roost locations were placed on 1998 3.75 minute Digital Orthophoto quarter quadrangle aerial photography obtained from the USGS. The 1998 photography was compared to versions of USGS mapping used during earlier years of the study to ensure consistency. Aerial photographs were placed in ArcView™ geographic information system software. Habitat within 1.6 and 4 km of each primary and alternate maternity tree was characterized to 1 of 5 habitat types, based on the Anderson et al. (1967) land cover classification system: agriculture (including grasslands), developed, forested, scrub-shrub, and water. The 4 km radius was used because it is frequently applied during regulatory concerns for the species and represents a distance equal to or greater than the average of most documented movements. The 1.6 km radius was used for comparison and was a distance sometimes used for regulatory issues in the past. Areas of each habitat type were tabulated using GIS.

RESULTS

No Indiana myotis were caught in 1987 or 1994, 4 (3 females and 1 male) were caught in 1997; 4 in 1998 (2 females and 2 males); and 3 in 2003 (2 lactating females and a juvenile).

Capture and roost sites.—Indiana myotis were captured at three sites: 2 bats at both Sites II and III in 1997, 3 bats at Site I and 1 bat at Site III in 1998, and 2 bats at Site II and 1 bat at Site III in 2003 (Fig. 2). Distances among capture sites were 3.1–5.9 km (Table 1). In 1997, Indiana myotis were radio-tracked to two primary roosts, #213 and #380 (Fig. 2), monitored in 1997 and 1998. In 2003 Indiana myotis were tracked to two primary (#407C and #708) and two alternate roosts (#407A and #407B; Fig. 2). Roost #708 was in the same woodlot and near roost tree #380, but that roost could not be precisely relocated in 2003. Distances among primary roosts were 2.8–5.5 km, while distances among all roosts were <0.1–5.5 km (Table 1). Distances among all capture sites and all roosts were <0.1–6.3 km ($\bar{X}$ = 3.2; *SD* = 2.7).

All roosts were behind exfoliating bark; all primary roost trees were dead and the two alternate roost trees were living (Table 2). Roost #213, a sugar maple (*Acer saccharum*), had been girdled in 1993 for timber stand improvement. Canopy closure over primary roosts (0–15%) was less than over alternate roosts (45–90%). Primary roost trees were larger ($\bar{X}$ = 53.3 dbh; *SD* = 31.2) than alternate roost trees (30 and 35 cm dbh).

Primary roost trees housed 9–50 bats during the period 1 June–25 July. In 1997, the number of bats in two roosts increased from 33 on 28 June, probably shortly before parturition, to 50 on 31 July when young are volant, although some adult females may have roosted at alternate locations by this later date. Similarly, the number of bats in roost #380 increased from 7 to 13 during the period 23 June–17 July 1998. Roosts #708 and #407C (9–11 bats and 21–35 bats, respectively) were observed in 2003 when young would have been volant, and some females may have left maternity roosts.

Roost #213 was along the southern edge of a 331 ha woodland that was part of a woodland corridor along Jonathon Creek. Primary roosts #380 and #708 were along the southern edge of a 331 ha woodlot surrounded by agricultural lands and roads (U.S. Route 63 to the east and a two-lane paved road to the north). Across the two-lane road and extending north was a wooded unnamed drainage.

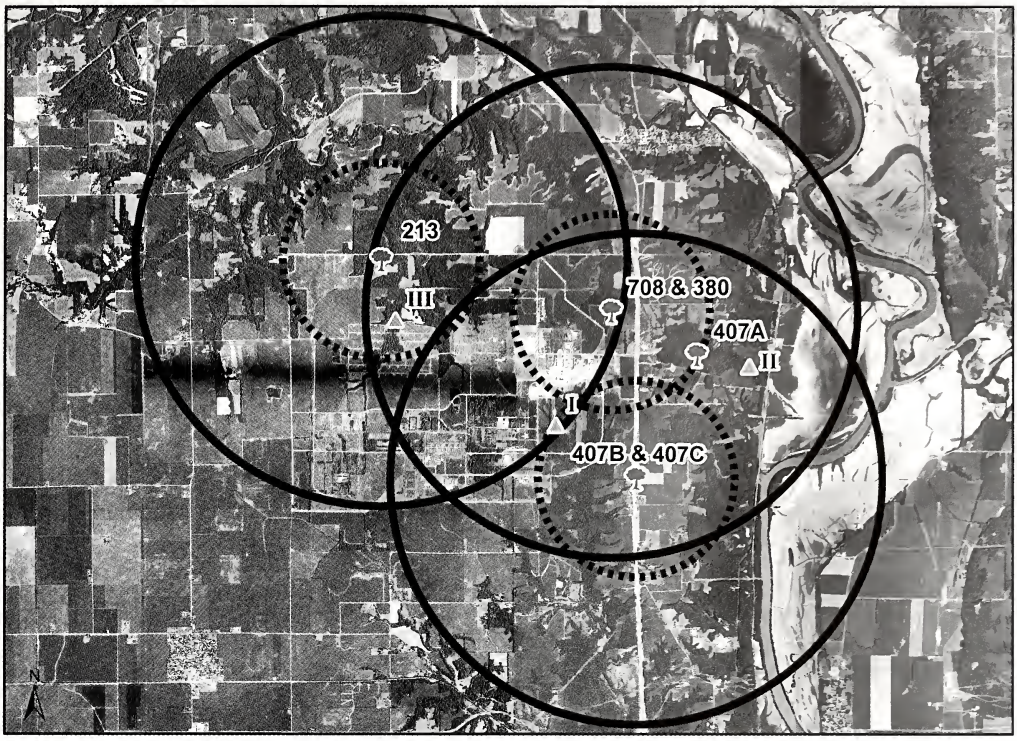

Figure 2.—Aerial photograph of capture sites (I–III, denoted with closed triangles), roost trees (denoted by a tree icon and labeled with a numeral), and the surrounding landscape on Newport Chemical Depot, Vermillion County, Indiana. The circles formed by dotted and solid lines are 1.6 km and 4.0 km around primary maternity roosts.

Roost #380 was a slippery elm (*Ulmus rubra*) 51 cm dbh and roost #708 was an American elm (*Ulmus americana*) 34 cm dbh (Table 2), while dominate trees near them were 25–30 cm dbh American elm, black locust (*Robinia pseudoacacia*), and black walnut (*Juglans nigra*).

Primary roost #407C and alternate roost #407B were in a small (0.7 ha) isolated woodlot. It was bounded on the west by a four-lane divided highway (U.S. Route 63) and by agriculture fields to the north, east, and south. The closest woody habitat was a brushy fence row of small trees west of Highway 63. The width of cleared highway right-of-way was about 30 m. The fence row, ultimately attached to the Little Raccoon Creek drainage, crossed open field for 660 m. Alternatively, the distance southwest to a wooded drainage of Little Raccoon Creek was about 180 m.

Alternate roost #407B bordered the U.S. Route 63 right-of-way and primary roost

Table 1.—Distances (km) among mist net capture sites (I, II, and III) and roosts (numerals prefaced by a # sign). Superscripts designate roosts as primary (P) or secondary (S).

	Site I	Site II	Site III	#213	#380/#708	#407A	#407B
Site II	3.3						
Site III	3.1	5.9					
#213^{P}	3.8	6.3	1.1				
#380/#708^{P}	1.9	2.5	3.6	3.8			
#407A^{S}	2.5	0.9	5.0	5.4	1.6		
#407B^{S}	1.6	2.6	4.7	5.5	2.7	2.2	
#407C^{P}	1.6	2.6	4.7	5.5	2.8	2.2	<0.1

Table 2.—Characteristics of and numbers of bats exiting four primary (multiple bats) and 2 alternative roost (single bat) trees used by Indiana myotis at Newport Chemical Depot, Vermillion County, Indiana.

Roost	Tree species	Status	dbh (cm)	% of bark exfoliated	% canopy closure	Height of roost (m)	Date observed	No. of bats
#213	*Acer saccharum*	Dead (girdled in 1993)	98	>25	0	7.6	6 June 1997	22
							11 June 1997	21
							18 June 1997	20
							28 June 1997	33
							31 July 1997	50
							1 June 1998	9
							26 June 1998	16
							17 Aug. 1998	0
#380	*Ulmus rubra*	Dead	51	>25	0	≈6.0	11 June 1997	16
							22 June 1998	5
							23 June 1998	7
							17 July 1998	13
							9 Sept. 1998	0
#407A	*Carya ovata*	Live	30	Yes*	45	6.1	21 July 2003	1
#407B	*Gleditsia triacanthos*	Live	35	Yes*	90	7.6	22 July 2003	1
#407C	*U. americana*	Dead	30	Yes*	10	12.2	23 July 2003	21
							24 July 2003	23
							25 July 2003	35
#708	*U. americana*	Dead	34	Yes*	15	10.7	21 July 2003	11
							22 July 2003	9
							23 July 2003	9
							24 July 2003	9
							25 July 2003	10
	Mean		46.3		26.7	8.4		14.8
	SD		26.5		35.2	2.5		12.3

* Exfoliation was observed but not recorded as a percent.

Table 3.—Percentages of five types of habitat within 1.6 and 4.0 km of primary roost trees.

Habitat type	1.6 km		4.0 km	
	$\bar{X}$	*SD*	$\bar{X}$	*SD*
Agriculture	44.0	5.4	46.6	3.1
Developed	11.0	6.8	11.4	0.8
Forest	39.5	17.1	35.6	3.0
Scrub shrub	5.4	6.3	5.6	0.2
Water	0.1	0.1	0.8	0.7

#407C was about 20 m east of the right-of-way, both on the west edge of the woodlot. The transmittered bat switched between the two roosts during daylight hours. Dominant trees near roosts #407B and #407C were 25–30 cm dbh honeylocust (*Gleditsia triacanthos*), black walnut, and sugar maple; primary roost #407C was 30 cm dbh and alternate roost #407B was 35 cm dbh (Table 2).

Landscape habitat characterization.—Habitat within 1.6 and 4.0 km of primary roosts was similar (Table 3). Developed lands were about 11% of the landscape and agricultural lands were about 45% of lands near primary roosts. Forested lands were 35–40% of the landscape. Within 1.6 km of a roost, about 321 ha (*SD* = 139) of wooded habitat was available, and within 4.0 km about 1811 ha (*SD* = 154) was available, however, because of overlap among areas (Fig. 2), total available woodlands was 0.6% less than individually within 1.6 km, and 49% less within 4.0 km (i.e., collective availability within 4.0 km of all three roosts was 3693 ha). Combined, scrub shrub and water were about 6% of the landscape.

DISCUSSION

The presence of multiple roost trees and multiple capture sites productive over a seven-year period, all within close proximity to one another, and movements of bats among roost trees within this area is strong evidence that a single maternity colony is using this area, and these data support the hypothesis of Kurta et al. (2002) that the Indiana myotis has a fission-fusion social organization. In Michigan, Kurta et al. (2002) found that members of one nursery colony used roosts up to 9.2 km apart over a four-year period, up to 8.2 km during a single season, and individual bats moved 5.8 km in a single night and 7.8 km in four days. At the Indianapolis International Airport during the period 1997–2001, a single nursery colony used four primary and 24 alternate roosts in a much larger area than that studied at Newport Chemical Depot (JOW pers. obs.). At Newport Chemical Depot we found four primary and two alternate roosts within 5.5 km of each another during three summers over a seven-year period. Thus, members of a maternity colony of the Indiana myotis roost singly, in small communal roosts, and in large communal roosts.

Many studies of the Indiana myotis emphasize natural features of forested areas where roosts are found (Callahan et al. 1997; Carter & Feldhamer 2005; Kurta et al. 2002), although at a landscape scale, these areas are dominated by human activities. Studies addressing the influence of humans on the habitat used by this bat are few (Sparks et al. 2005). Within the core summer range, woodlands are fragmented by agriculture and urbanization; only 24% of land in counties with records of reproductive Indiana myotis is forested (Gardner & Cook 2002). Only 20% of Indiana is forested (Tormoehlen et al. 2000) and many counties in the northern two-thirds of the state are <10% forested (Schmidt et al. 2000). Habitat of the Newport Chemical Depot study area, about 35–40% forested, was nevertheless fragmented, with bats using isolated woodland parcels. Before European settlement, the core range (including the study area) was a mosaic of woods and prairies, oak-dominated savannahs, and wetlands including marshes and open swamps (Brugam & Patterson 1996; Homoya et al. 1985; Nuzzo 1985). Thus, woodlands were naturally fragmented in the past, while today fragmentation is a result of agriculture and development.

Regardless of the cause, the Indiana myotis is adapted to using a fragmented landscape, which would necessitate crossing open, unforested areas. Relying upon radiotelemetry, Murray & Kurta (2004) found that members of a colony of Indiana myotis followed a tree line rather than cross an open agricultural area. They interpreted this as a reluctance of the species to cross open lands. In this study, it is obvious bats crossed a variety of open lands, including agricultural lands and a divided four-lane highway. Brack (1983) reported light-tagged individuals crossing open pastures. One author of this paper (VB) has

caught Indiana myotis in a variety of open habitats including over a ditch (channelized stream) in an open agricultural bottom land in southern Illinois, in open pipeline corridors in predominately wooded areas of New York, and over open roads in many portions of the range. We suspect bats follow tree lines, fly over ditches in open fields, and follow openings through woodlands because they use these landscape features for navigation.

Indiana bats have been caught, observed, and radio-tracked foraging in open habitats (Brack 1983; Clark et al. 1987; Gumbert 2001; Hobson and Holland 1995; Humphrey et al. 1977; Sparks et al. 2005), which is supported with studies of food habits, including at least one incidence of a Hessian fly (*Mayetolia destructor*, a pest on wheat) in the diet (D. Sparks pers. comm.). In Indiana, individuals foraged most in habitats with large foliage surfaces, including woodland edges and crowns of individual trees (Brack 1983). Woodlands with open canopies, including wooded pastures and recently logged areas, provide a greater foliage area for foraging than woodlands with a closed canopy. Woodland openings provide more insects than woodland interiors (Tibbels & Kurta 2003). Many woodland bat species forage most along edges, an intermediate amount in openings, and least within forest interiors (Grindal 1996).

It might seem that Indiana bats should avoid open areas in order to avoid predators such as owls. However, owls seldom prey on bats (Sparks et al. 2000). Instead, they prey mostly on small and medium-sized mammals, hunting by listening for sounds made as they move about on the ground. Possibly the few bats eaten by owls are disabled or are on the ground. If owls use eyesight to capture bats, then bats should avoid open areas at times of greater lunar illumination. However, to date there is no evidence of such avoidance by bats from temperate regions (Parsons et al. 2003).

The Indiana myotis roosts in trees with sloughing bark that are typically large, often dead or dying, and typically exposed to solar heating. Three of four primary roosts on Newport Chemical Depot were on the southern edge of woodlands and the fourth was on a western edge. Thus, the sun would warm all primary roosts; and canopy closure was minimal. Kurta et al. (1993) found that all of eight roosts were exposed to direct sunlight throughout the day, and both Kurta et al. (2002) and Carter & Feldhamer (2005) documented numerous roosts exposed to abundant solar radiation.

Managers of publicly-owned wooded lands, federal and state forests, parks, wildlife management areas, and Dept. of Defense facilities have developed management plans to conserve wooded habitat for this endangered bat. Within the fragmented landscape of the core range, preservation of wooded drainages and isolated woodlots will benefit the species. While beneficial, conservation of large tracts in the core range is difficult because open areas are in agriculture and expensive to acquire. However, conservation areas need not be large or contiguous to benefit the bat. Creating small wooded areas and enlarging existing small areas within proximity to one another, should benefit the species, and in some cases may be the only way that enough habitat can be maintained on the landscape to support a colony. In contrast, much of the eastern portion of the range of the Indiana myotis is heavily wooded, and yet the species is less common. Brack et al. (2002) noted that more trees do not mean more bats; and in the eastern United States, removal or replacement of smaller tracts of woodland in areas of abundance may affect the species less.

In summary, in many portions of Indiana, the Indiana myotis uses isolated woodlots and wooded drainages on an agricultural landscape. Use of several roosts by a maternity colony supports the hypothesis of a fission-fusion social system. Movement across open spaces among habitat patches may readily occur because the species is adapted to a natural interspersion of open and wooded habitats. Loss or conservation of small or isolated wooded tracts within the core range may harm or benefit the species disproportionately more than in other portions of the range.

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NESTING SUCCESS AND STATUS OF THE LEAST TERN BREEDING COLONY AT GIBSON LAKE IN SOUTHWESTERN INDIANA

Travis L. DeVault and **Michael B. Douglas**: Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 47907 USA

John S. Castrale: Indiana Division of Fish and Wildlife, Mitchell, Indiana 47446 USA

Charles E. Mills: 8600 Framewood Drive, Newburgh, Indiana 47630 USA

Timothy Hayes: Cinergy Corp., 100 East Main Street, Plainfield, Indiana 46168 USA

Olin E. Rhodes, Jr.: Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 47907 USA

ABSTRACT. Federally-endangered interior least terns (*Sterna antillarum*) have nested at Gibson Lake in southwestern Indiana since 1986. In 2003 and 2004 we evaluated nesting success of least terns and identified factors influencing their productivity at Gibson Lake. In 2003 least terns nested only at secondary breeding sites, composed of deposited ash byproducts from coal combustion. Least terns produced eight fledglings from 39 total nests in 2003; we attributed most nest failures that year to flooding during heavy rains. In 2004 the terns returned to breed at their historical primary breeding site, a narrow gravel dike that bisects Gibson Lake (center dike). However, nesting success again was low in 2004 (13 fledglings produced from 84 total nests), although we attributed most nest failures in 2004 to predation on eggs and chicks by ring-billed gulls (*Larus delawarensis*). Our study implicated ring-billed gulls as the chief source of mortality on least tern eggs and chicks. The future success of the least tern breeding colony at Gibson Lake ultimately may depend on the establishment and sustained management of alternate breeding areas in the Gibson Lake vicinity.

Keywords: Indiana, *Larus delawarensis*, least tern, nest success, productivity, ring-billed gull, *Sterna antillarum*

Least terns are robin-sized waterbirds that nest colonially on sandy coastal beaches, river islands, and artificial structures such as sand pits, ash disposal areas, and gravel rooftops (Thompson et al. 1997). Gibson Lake in southwestern Indiana has been used as a nesting site by least terns (*Sterna antillarum*) since 1986 (Mills 1987; Castrale et al. 1999). Preferred nest sites of the least tern are largely free of vegetation, above high water levels, and protected from ground predators (Thompson et al. 1997).

Populations of least terns declined rapidly in the early 1900s, chiefly due to exploitation by overzealous feather and egg collectors. The interior population, which breeds in the central U.S. along major river systems, further declined in the mid-1900s because of river damming, channelization, pesticide use, and human development (Thompson et al. 1997; Kirsch & Sidle 1999). The interior population of the least tern was listed as federally-endangered in 1985 (Federal Register 1985) because of low population numbers and continued threats to breeding habitats (Kirsch & Sidle 1999). In addition, nest predators often limit productivity of least terns breeding in both natural and artificial sites (Kruse et al. 2001; DeVault et al. 2005).

The least tern colony at Gibson Lake is an important component of the interior population, and potentially could serve as a source of dispersing individuals for colonies along the Wabash and Mississippi rivers. In spring 2003 we initiated research aimed at evaluating the nesting productivity (hatching and fledg-

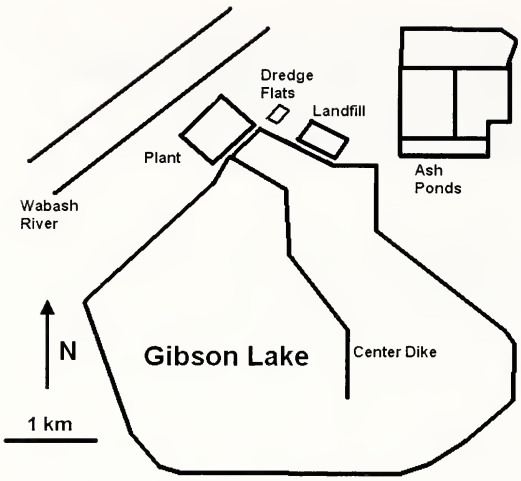

Figure 1.—Schematic of Gibson Lake and surrounding features of the Gibson Generating Station in Gibson County, Indiana. The center dike, dredge flats, landfill, and ash ponds are nesting areas for least terns. See the text for a description of the nesting areas.

ing success) of the least tern colony at Gibson Lake. In a previous paper (DeVault et al. 2005), we reported on our efforts using remote cameras aimed at model nests and actual least tern nests to identify nest predators at the Gibson Lake tern colony. We demonstrated that ring-billed gulls (*Larus delawarensis*) were responsible for most egg and chick mortality. Here, we further explore the relationship between ring-billed gulls and least tern nesting success at Gibson Lake by relating gull abundance to predation on least tern eggs and chicks. We also report on the productivity of the least tern colony, focusing on the breeding seasons of 2003–2004. Castrale et al. (1999) updated the status of the Gibson Lake tern colony from its inception in 1986 through 1998.

STUDY AREA AND HISTORY OF THE LEAST TERN COLONY AT GIBSON LAKE

The Gibson Generating Station, owned and operated by Cinergy Corp., is a large, coal-fired power generating station located in Gibson County in southwestern Indiana, adjacent to the Wabash River (Fig. 1). A principal feature of the power plant is Gibson Lake, a shallow (depth averaging 4 m) 1180 ha impoundment that cools water during the generation of electricity. Gibson Lake contains large populations of small fish, including threadfin shad (*Dorosoma petenense*), gizzard shad (*Dorosoma cepedianum*), and brook silverside (*Labidesthes sicculus*). A narrow (~10 m wide), 3.4 km long dike (center dike), composed of crushed limestone gravel with larger riprap along the edges, bisects the lake (Fig. 1).

The breeding colony at Gibson Lake is the eastern-most established colony known to exist for interior least terns (Kirsch & Sidle 1999). Least terns first began nesting at Gibson Lake in 1986 (Mills 1987), when a single pair was found nesting on the center dike (Fig. 2). Since that time, least terns have nested at Gibson Lake every year except 1988. The center dike has been the primary nesting site during most of the history of the Gibson Lake least tern colony, although three other areas (ash ponds, landfill, and dredge flats; Fig. 1) near Gibson Lake have been used as secondary nesting sites in recent years. The three secondary nesting habitats are composed of deposited ash byproducts from coal combustion, although they vary with respect to the specific type of ash present, topography, and potential for flooding during heavy rains. Least terns first began nesting on the landfill in 2003 and on the dredge flats in 2004. The ash ponds and surrounding roads have been used for nesting by least terns periodically since 1993.

The least tern colony at Gibson Lake has been managed since its inception through cooperative agreements between the Indiana Department of Natural Resources, the U.S. Fish and Wildlife Service, and Cinergy Corp. Several management activities have been implemented on the center dike to make it more attractive to nesting least terns, including the use of decoys to attract nesting individuals, the regular use of herbicide to prevent overgrowth of vegetation, and strict control of human access during the breeding season (Johnson & Castrale 1993).

Many predators of least tern eggs and chicks (Thompson et al. 1997), such as ring-billed gulls, herring gulls (*Larus argentatus*), great blue herons (*Ardea herodias*), black-crowned night-herons (*Nycticorax nycticorax*), American crows (*Corvus brachyrhynchos*), and various hawks and owls, occupy the Gibson Lake area. Mink (*Mustela vison*) and Norway rats (*Rattus norvegicus*) have

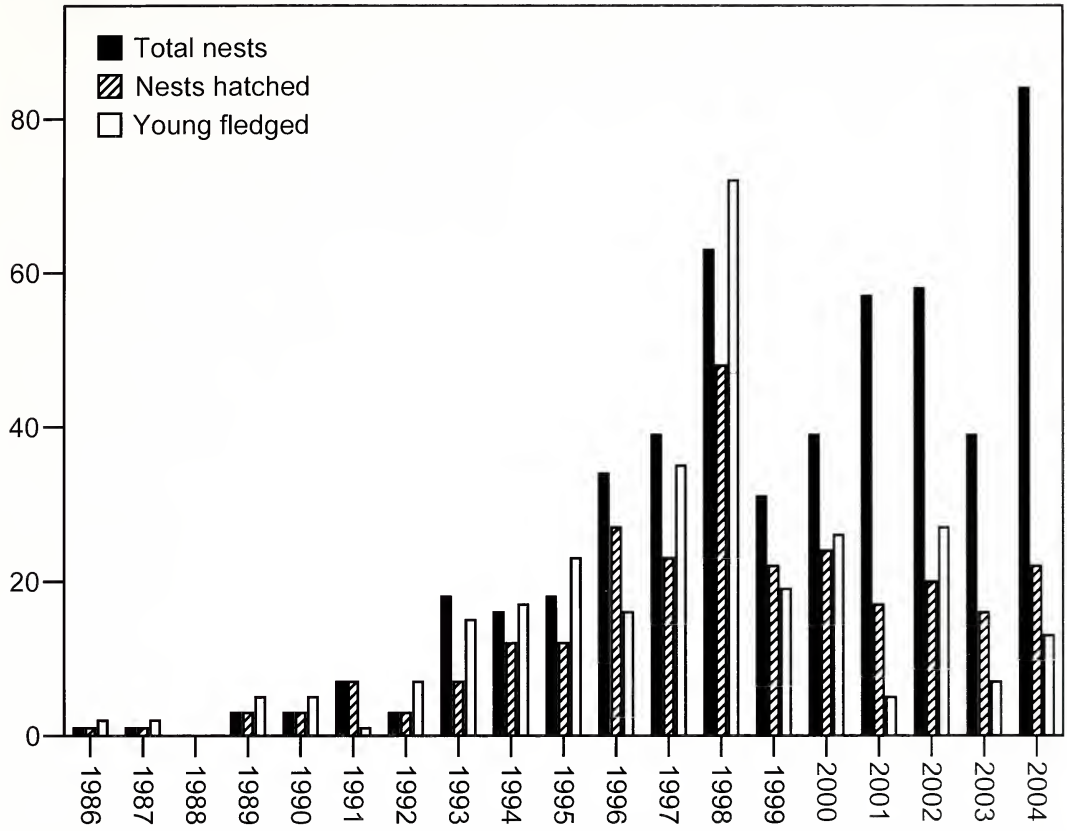

Figure 2.—Nesting productivity of least terns at Gibson Lake, Indiana, from 1986–2004. Hatched bars indicate the number of nests in which at least one egg hatched; open bars indicate the number of chicks that fledged from all successful nests.

been present on the center dike, and raccoons (*Procyon lotor*) and Virginia opossums (*Didelphis virginiana*) are common throughout the Gibson Lake area. A chain-link fence is located near the north end of the center dike to prevent medium-sized mammalian predators easy access to the colony. Also, there have been periodic efforts to control nest predation on the center dike, such as the use of strobe lights to reduce nocturnal predation by great horned owls (*Bubo virginianus*) and black-crowned night-herons, the construction of plywood chick shelters and open-top predator-exclusion fences around individual nests, and lethal control of mink, Norway rats, raccoons, and ring-billed gulls (Johnson & Castrale 1993; DeVault et al. 2005).

Throughout most of the 1990s the Gibson Lake least tern colony was one of the most productive colonies in the interior population (Castrale et al. 1999; Kirsch & Sidle 1999; Fig. 2). As demonstrated in 1998 (72 fledglings from 63 total nests), the Gibson Lake colony has the potential to produce a substantial number of fledglings (Fig. 2). However, beginning in 1999, the nesting success (percentage of nests from which at least one chick fledged) of least terns at Gibson Lake dropped below 40%, and in 2001, nesting success dropped to only 5%. Although the number of nesting attempts by least terns at Gibson Lake increased from 31 in 1999 to 58 in 2002, nesting success averaged only 27% during that time period. In contrast, from 1995–1998, nesting success at the colony averaged 51%, with a high of 70% in 1998. The sharp decrease in productivity in recent years most likely can be attributed to increased predation on eggs and chicks (JSC unpubl. data). Although quantitative data are lacking, anecdotal observations have suggested that ring-billed gulls, in particular, have increased in number

at Gibson Lake during the years that least tern nesting success declined (JSC unpubl. data). We strongly suspect that ring-billed gulls are attracted to fish carrion that regularly accumulates around the center dike.

METHODS

Nesting productivity.—During the breeding seasons of 2003 and 2004 we visited all potential least tern nesting sites approximately every two days to search for new nests and to check the status of extant nests. Because the substrate was extremely unstable at the dredge flats, we were unable to reach those nests on foot; instead, we observed least terns nesting at the dredge flats with a spotting scope from ~50 m. At all other sites, we approached the colony on foot and recorded the position of each nest with a mobile Global Positioning System unit. During visits to nesting areas we checked all extant least tern nests for evidence of predation or abandonment. When they were 1–3 days old, we banded each least tern chick we encountered with one plastic and one aluminum leg band.

Gull surveys.—Surveys of ring-billed gulls were conducted from 20 May through 28 August in 2003 and from 15 April through 19 August in 2004 (see also DeVault et al. 2005). We conducted approximately three surveys during each week, although we postponed surveys during inclement weather. During each survey, we slowly drove around Gibson Lake and throughout the remainder of the Gibson Generating Station property (including the length of the center dike) and recorded the number of ring-billed gulls observed. After least terns began nesting on the center dike in 2004, automobiles were prohibited on the dike and we instead conducted gull surveys on foot when checking the status of nests. We conducted most (> 90%) gull surveys before 0900 h.

Statistical analyses.—For the 2004 nesting season, we used linear regression to explore the relationship between the number of ring-billed gulls roosting on the center dike and predation pressure on least tern eggs and chicks at the center dike. The number of ring-billed gulls roosting on the center dike during each consecutive one-week period of the breeding season was estimated by calculating the mean number surveyed on the center dike during the one-week period (we normally conducted three surveys per week). We calculated predation pressure during each one-week period as the number of predation events on nests at the center dike recorded during each week divided by the total number of least tern nests available to predators on the center dike during that week. Analyses were performed with SPSS version 10.0 (SPSS 1999).

RESULTS

Bird surveys.—We observed a steady increase in the number of ring-billed gulls occupying the center dike during the 2003 breeding season. The number of gulls ranged from 25 individuals to over 250 near the end of the breeding season (DeVault et al. 2005). In 2004 there were fewer than 20 ring-billed gulls on the center dike from 10 May through 20 June. The relatively low number of ring-billed gulls on the center dike at the beginning of the breeding season in 2004 can be attributed to lethal removal (by shooting) of gulls in April, before least terns began nesting (DeVault et al. 2005). However, during the period 20 June to 1 August, the number of ring-billed gulls occupying the center dike rose steadily, mirroring counts from 2003 (DeVault et al. 2005).

Nesting productivity.—For the first time since 1989, least terns did not nest on the center dike at Gibson Lake in 2003. Least terns nested at the ash ponds and the landfill from 6 June through 24 July. At the ash ponds, at least one egg in 12 of 19 total nests survived to hatching, and eight chicks survived to fledging. The unsuccessful nests were lost to predation and abandonment at the ash ponds. At the landfill, at least one egg in 4 of 20 total nests survived to hatching, but no chicks survived to fledging. All nests that failed to produce chicks at the landfill were washed away during heavy rains. Least terns did not nest at the dredge flats in 2003.

In 2004, least terns constructed 84 nests from 30 May through 15 July. Seventy-two nests were constructed on the center dike and twelve were constructed at the dredge flats. There was very little exposed substrate at the ash ponds in 2004, which precluded nesting by least terns. No least tern nests were found at the landfill in 2004.

On the center dike, at least one egg in 16 of the 72 nests constructed in 2004 survived to hatching. Four chicks fledged from two

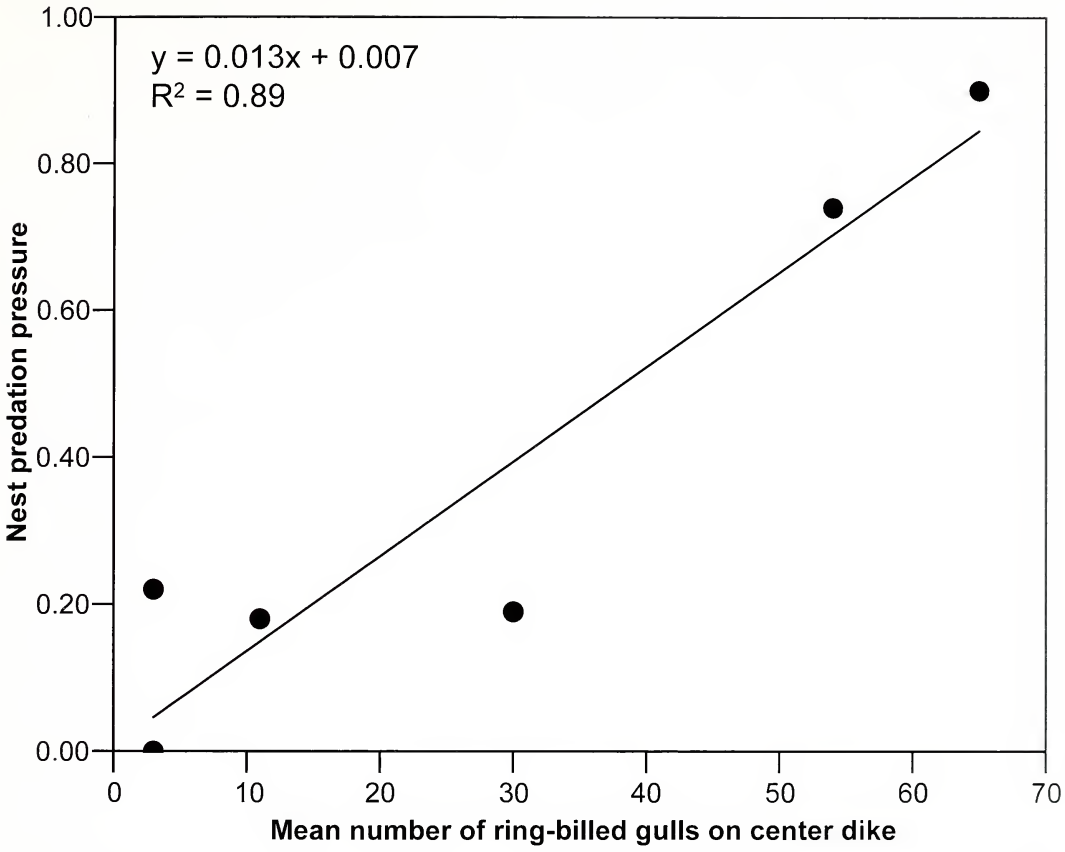

Figure 3.—Predation pressure on least tern nests on the center dike of Gibson Lake, Indiana, plotted as a function of the mean number of ring-billed gulls surveyed on the center dike. Points represent seven consecutive one-week periods from 1 June 2004 through 19 July 2004. Nest predation pressure was calculated as the number of predation events recorded during the one-week period on the center dike divided by the number of least tern nests available to predators on the center dike at some time during the week. Note that two data points occupy the point at the bottom of the graph (0 nest predation pressure; 3 ring-billed gulls).

successful nests on the center dike. Least tern hatching success on the center dike was much higher during the first 10 days of the nesting season. During the period 30 May through 10 June, 14 of the 23 nests (61%) on the center dike in which eggs were laid survived to hatching; however, only 2 of 49 nests (4%) in which eggs were laid after 10 June survived to hatching. No eggs laid after 20 June successfully hatched. Nest failure at the center dike was attributed primarily to nest predation, and nest predation pressure was strongly correlated with the number of ring-billed gulls on the center dike (Fig. 3). On the dredge flats, nine chicks fledged from four successful nests. All eight nest failures at the dredge flats were due to flooding.

Five leg bands from least tern chicks were recovered (all on the center dike): one was found in a regurgitated pellet (presumably from a ring-billed gull), and three chicks were found dead from unknown causes at nests. Additionally, one band was found lying on the ground at a nest.

DISCUSSION

One of the most convincing results of our research was that ring-billed gulls serve as the primary predators of least tern eggs and chicks on the center dike of Gibson Lake. Data from remote cameras indicated that other species, such as American kestrels (*Falco sparverius*), snakes, and Canada geese (*Branta canadensis*), occasionally prey upon or oth-

erwise damage least tern eggs and chicks, but ring-billed gulls almost certainly were responsible for most least tern egg and chick mortality at Gibson Lake in 2004 (DeVault et al. 2005).

Our data indicated that the number of ring-billed gulls present on the center dike was strongly correlated with the probability of nest predation on the dike. There were few instances of predation on least tern eggs during the first 10 days of the breeding season in 2004, when ring-billed gulls were scarce. However, ring-billed gulls gathered at Gibson Lake, and particularly at the center dike, over the course of the breeding season; and by late June in 2004, the population of ring-billed gulls on the center dike had grown to a size that apparently precluded successful nesting by least terns (Fig. 3; see also DeVault et al. 2005). Thus, a reduction of ring-billed gulls on the center dike likely would increase the productivity of least terns at Gibson Lake, but only if the number of ring-billed gulls occupying the center dike could be maintained at a minimum (< 20 individuals) during the entire breeding season.

Although predation by ring-billed gulls on least tern eggs and chicks was the primary source of mortality on the center dike, we recorded relatively few predation events at the three secondary nesting areas. In 2003, breeding least terns were moderately successful at the ash ponds (eight fledglings from 19 nests); and although no fledglings were produced at the landfill, the primary source of mortality at that site was flooding, not predation. Similarly, in 2004, nine chicks fledged from four successful nests at the dredge flats; and all mortality at that site also was caused by flooding. Although it is difficult to control mortality from flooding, it may be possible to deter least terns from nesting in areas where they have a low probability of producing fledglings (i.e., the landfill) using a suite of fear-provoking stimuli (Conover 2002).

The least tern colony at Gibson Lake has continued to grow in terms of the number of nests constructed (Fig. 2), but the colony undoubtedly will serve only as a population sink (Pulliam 1988) or reproductive trap (Gates & Gysel 1978; Battin 2004) unless a higher percentage of nests begin to produce fledglings. It now appears evident that least terns breeding on the center dike will continue to suffer from substantial nest predation unless numbers of ring-billed gulls can be controlled. Unfortunately, the logistic difficulties and ethical issues associated with lethal removal of large numbers of gulls lessen the ability to successfully manage the center dike as a nesting area for least terns at Gibson Lake. Also, nest disturbance techniques (e.g., Ickes et al. 1998) are not viable because ring-billed gulls do not breed at Gibson Lake (most present are juvenile loafers). Thus, the ultimate solution for effective conservation of least terns in the Gibson Lake vicinity likely will involve the establishment and sustained management of alternate nesting habitats.

Extensive efforts are underway to increase the available nesting habitat for least terns on sites adjacent to Gibson Lake. Two properties, the Cane Ridge Wildlife Area (owned and managed by the U.S. Fish and Wildlife Service) and Tern Bar Slough (owned and managed by the Indiana Department of Natural Resources) are being developed to provide new nesting habitats for least terns. At the Cane Ridge Wildlife Area, a 24 ha impounded least tern nesting unit containing a 1.2 ha nesting island, was constructed recently. This nesting unit will be operated and managed specifically for nesting least terns, and managers will attempt to attract least terns to the nesting unit with decoys and vocalizations (Johnson & Castrale 1993). Tern Bar Slough is approximately 340 ha, and plans are being developed to include least tern nesting islands during the restoration of that property.

Least terns began nesting at the Cane Ridge Wildlife Area during the breeding season of 2005 and appear to be producing fledglings at a high rate. Thus far, ring-billed gulls have not been present in significant numbers at Cane Ridge. We anticipate that ring-billed gulls will not roost commonly at Cane Ridge or Tern Bar Slough, and thus will not be a major source of mortality to least tern eggs and chicks at those locations. Ring-billed gulls were observed only rarely at sites other than the center dike during our bird censuses in 2003 and 2004. Furthermore, during experiments with remote cameras at the ash ponds and landfill, we recorded no instances of predation on model nests or actual least tern nests by ring-billed gulls (DeVault et al. 2005). We expect that the center dike will continue to be the preferred roosting site for ring-billed gulls

in the Gibson Lake vicinity because of its isolation to land and because of the reliable food source present there in the form of fish carrion (DeVault et al. 2005). However, if ring-billed gulls prove to be a major source of mortality to least tern eggs and chicks at the Cane Ridge Wildlife Area or Tern Bar Slough, other management techniques should be evaluated, such as the removal of gulls by trapping and the use of fear-provoking stimuli to repel gulls from least tern nesting areas.

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MARION THOMAS JACKSON: A LIFELONG NATURALIST, CONSERVATIONIST, AND EDUCATOR

John O. Whitaker, Jr.: Department of Ecology and Organismal Biology, Indiana State University, Terre Haute, Indiana 47809 USA

Editor's note: This is the second in a series of biographical articles honoring notable living members of the Indiana Academy of Science. These scientists have all served the Academy with distinction and have made valuable contributions in their respective fields of scientific inquiry. We have chosen Dr. Marion Thomas Jackson, Professor Emeritus in the Department of Ecology and Organismal Biology at Indiana State University, as the subject of this second installment in this series.

I am very pleased to have been asked by Jim Berry to write an article in this biographical series about Marion T. Jackson. I have known Marion since August 1964 when he first arrived at Indiana State University as an Assistant Professor of Ecology. Marion has been my friend and colleague for more than 40 years. In addition to team teaching on occasion, he and I typically serve on the graduate committees of each other's students. I often went to Marion for advice and ideas. He routinely offered helpful suggestions regarding my manuscripts, as he is among the best editors that I have ever known.

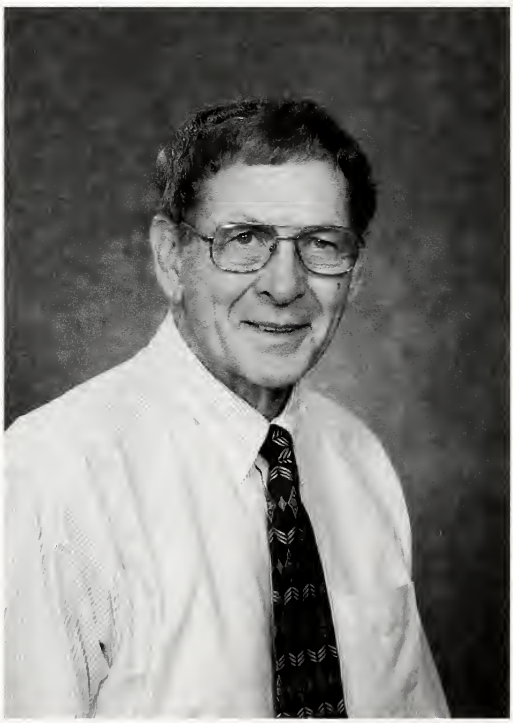

Figure 1.—Marion Jackson, Professor Emeritus at Indiana State University.

THE EARLY YEARS AND PERSONAL LIFE

Marion Thomas Jackson was born on 19 August 1933, on a 160-acre farm located 1½ miles southeast of the town of Versailles in Ripley County, Indiana. He was the seventh child, and fourth son, of Marshall Marion and Estella Fox Jackson. His birthplace was purchased by the State of Indiana in 1936, for inclusion within the proposed Versailles State Park. Laughery Bluff Nature Preserve now contains the old-growth beech-maple stand that formerly was the Jackson farm forest.

The farm in the Tanglewood Community, located three miles southwest of Versailles, where Marion grew up, was homesteaded by his grandfather's Grandfather Laswell in the 1820s. Marion's family moved there in 1937, and the home farm is still owned and farmed by an older brother. During the late 1930s and the 1940s, when Marion was a boy, the land was farmed with horses, as a general purpose farm of that era.

The diversified rolling landscape, with small creeks, fence rows, woods, quarry ponds, meadows, old fields, and crop fields farmed in rotation, was a haven for a lad interested in natural history from early boyhood. Furthermore, the family supplemented their

food supply by hunting, fishing, and gathering greens, berries, nuts, and other wild plant foods. Such activities enabled Marion to learn the natural history of most wild species by early grade school. Also, extremely valuable insights into land management, conservation of natural resources, and practical ecology were continually absorbed by the alert and inquisitive farm boy, as the family worked the land. Such understandings were to prove vitally important to him throughout his career.

A particular boyhood interest was searching for bird nests during times free from farm chores. Marion still has the small one-cent *Goldenrod* childhood tablets with his records of bird nests observed during the summers of 1943–1945.

Marion was President of his high school class during all four years and graduated at the top of his class in May 1951, a record good enough to qualify him for an Edward Rector Scholarship to DePauw University. But for reasons understood only by 17-year-olds, he elected not to enter college at that time. Instead, he worked for Coca-Cola in Fort Wayne, Indiana, for two years as Assistant Production Manager. He then served in the U.S. Naval Reserve during the Korean War, as an Electronics Technician aboard the USS Currituck, a seaplane tender in the Atlantic Fleet.

After discharge from active duty, Marion worked in the electronics industry (ITT) in Fort Wayne as a technical writer for three years. Then one blue-sky spring day, when the birds were singing, he decided that his long detour from the outdoors needed to end, and that he *had* to spend his life as a naturalist. As such, Marion entered college at Purdue University in June 1958.

Marion is married to Jaleh (née Jorjani), a registered nurse at Union Hospital in Terre Haute. They have two daughters, Arshia Myers (a dentist in Dayton, Ohio), and Grousha Birkenbeul (a science teacher in Fort Wayne), and three (soon to be four) granddaughters, aged 1½ to 3½, at this writing. Jaleh and Marion reside at 4207 E. Margaret Drive, Terre Haute, Indiana 47803, where they have lived for the past 21 years.

Besides his active career as a professional ecologist and naturalist, Marion greatly enjoys writing—both science and popular nature writing, visiting natural areas and national parks, reading, gardening, woodworking, photography, storytelling, visiting with friends and extended family, and playing with the granddaughters.

Figure 2.—Marion Jackson at age 10 at Versailles Grade School (April 1944).

THE PURDUE YEARS

Marion spent his freshman year at Purdue as a forestry major; but the strong emphasis on management, especially the "saw-log production" approach of that era, was not what he wanted as a career. When he took Dr. Alton Lindsey's Plant Ecology course as a sophomore, he found the discipline to be satisfying and intellectually stimulating. Marion had discovered his professional niche. Rather than change schools within Purdue, instead he changed his major to Conservation of Natural Resources, under the mentorship of Professor Howard Michaud. Marion graduated with a B.S. degree from Purdue's School of Forestry and Natural Resources with Highest Distinction in June, 1961.

When Dr. Lindsey agreed to accept Marion as a Ph.D. Student, application was made for a National Defense Education Act (NDEA) Title IV Fellowship to support his graduate education. The three-year fellowship funded the completion of all course work and field research of his study on the "Effects of Microclimate on Spring Flowering Phenology," which was carried out at Allee Woods, a research and teaching natural area owned by

Figure 3.—Jaleh and Marion Jackson at home (May 2004).

Wabash College, and located in Parke County, Indiana. Marion received his Ph.D. in Plant Ecology in June 1964.

Dr. Lindsey was a most fortuitous choice for a major professor because he was one of the foremost ecologists of the 20th Century. Additionally, he was a member of the Ecologists Union, the parent committee of ecologists within the Ecological Society of America that founded The Nature Conservancy. He also was the last surviving member of Admiral Byrd's 2nd Antarctic Expedition.

A tribute to Purdue faculty members and others who guided and greatly influenced Marion's career preparation is contained in a later section of this biography entitled "As the Twig is Bent."

"My Personal Philosophy"
Marion Jackson (2004)

Teaching as a profession is potentially immortal. The minds and lives that you touch today may ultimately influence those afar, or generations yet unborn. Those privileged to teach must not take their opportunity lightly. Human futures are too priceless to be entrusted to the unskilled, or those lacking commitment or conviction.

In many respects an academic career as a professor at a college or university is the finest of all professions. Helping educate young minds in preparation for their lifetime employment and contributions to humanity, surely ranks among the most rewarding ways that a person could earn their livelihood. Speaking for myself, there is no one in the State of Indiana with whom I would have willingly exchanged jobs. My personal philosophy is that all humans should strive to live their life in such a way that they make living conditions better for fellow humans, and also to improve the condition of Planet Earth.

Teachers at the college level must make every effort to help young people in their classes move forward along what I call the "S" Continuum, namely:

Stranger ↔ Spectator ↔ Student

↔ Scholar ↔ Scientist

Some who enroll in your class are *Strangers* who seldom come to class. Others show up, but are *Spectators* who rarely become involved in active learning. *Students* are the majority who study consistently in order to do well. Teaching at the advanced level is my opportunity to help *Students* strive to become true *Scholars*, or, for the occasional gifted and dedicated individual, to become a *Scientist.*

By helping gifted students formulate hypotheses, then design research studies to prove the rightness or wrongness of their premise, and finally, through systematic research, to become more expert on their chosen topic than perhaps any other person on Planet Earth, is teaching at its finest, and most rewarding. I love the process of taking raw-recruit students and helping them develop the skills that makes them true *Scholars* and *Scientists.*

HIS TEACHING

Marion is an excellent teacher—a Master Teacher. I have come to know of his teaching through discussions with many students who have taken his classes, through my own graduate students, through team teaching with him, and through working with him on a daily basis for 40 years.

Students often go out of their way to tell me how much they benefit from and enjoy his classes. He relates very well to students at all levels, and gives of himself to them—of his

Figure 4.—Ph.D. students working with Dr. A.A. Lindsey in Plant Ecology (Spring 1964). From left: Damian V. Schmelz (later at St. Meinrad College); William B. Crankshaw (later at Ball State University); Marion T. Jackson; John O. Sawyer, Jr. (later at Humboldt State University, California); Syed A. Qadir (later at University of Kurachi, Pakistan). Robert O. Petty (later at Wabash College) was not present for the photograph.

knowledge, his caring and concern, his humility, his competence, and his sense of humor. Students know he is doing all he can to help them in the classroom, and in later life.

My own graduate students usually want to take all of his classes. He makes them work, but he makes them think. He provides them with information about today's world, and he encourages them to use it for the betterment of humankind. I continually realize how really good he is with students, when my students seek his advice on their own, and I often send them to confer with him.

I have taught with him and know how he feels about students. He wants students to succeed; and aside from lowering his standards, he will do everything he can to help them succeed. In short, he loves students.

Two hallmarks of Marion's teaching have been group studies of environmental problems in his "Humans and World Environment" course, and extended field trips with advanced students in his "Biogeography" course during the past 40 years.

In his "Humans and World Environment" classes, students did investigations of either an urban, rural, or natural area of 20 acres or less in size. In each case an inventory was made of available resources, environmental problems were then assessed, followed by developing a set of recommendations regarding how the problems could be alleviated or solved. The finished product usually took the form of a written report ranging from 20–40 pages in length. The entire class worked jointly, both in the field collecting their data, and in the laboratory completing their report. This exercise gave them simulated real-life experience in identifying, analyzing, and solving environmental problems.

One class during the early 1970s that selected the ISU campus as their study area did an exceptionally fine job with their project. Consequently, Marion took the final report and the student recommendations for campus improvement to ISU President Richard Landini's office. Within a couple of weeks Dr. Jackson received a telephone call from Lan-

dini, stating that he had read the report with great interest and that he was forming a Landscaping Committee to be charged with making recommendations for campus improvement. He requested that Jackson chair the committee, and that some of the students in his class serve as committee members!

Extended field trips in "Biogeography" classes enabled his students to gain invaluable first-hand experience in understanding the determinants of distribution patterns and successes of plant and animal communities by conducting field studies in some of the most interesting and beautiful regions of our continent. Dr. Jackson truly makes ecology live in field settings.

In October of alternate years, for decades he took trips to the Great Smoky Mountains National Park, when the Eastern Deciduous Forest Biome is a blaze of color. The highlight of those trips was a day-long, 12-mile hike from the somber spruce-fir forests atop 6000-foot Mt. Collins, to the lush mixed mesophytic cove forests near Sugarlands Visitor Center. By plotting the elevation and location of the entry and exit of each forest tree species (from altimeter and topographic map readings—now, of course, GPS could be used), the elevational replacements of tree species could be plotted along their figurative "one-day ecological walk from Canada to Tennessee." Former students from 30 years ago still fondly reminisce about their Smokies experience in Dr. Jackson's "Biogeography" class.

Western North America was featured in late spring or summer trips to the southwest deserts, western coniferous forests, or to Alaska. Desert Ecology classes visited the Chihuahuan, Sonoran, Mojave, and Great Basin Deserts; whereas classes that focused on western forests visited 8–10 old-growth types ranging from Great Basin juniper-pinyon stands to giant sequoia-Sierra mixed, coastal redwoods, and Olympic cedar-hemlock forests. The main objective of these studies was to correlate density and diversity of breeding birds with differences in plant community structure and composition.

One summer (1970), Marion and Dr. Benjamin Moulton, Chairperson of Geography-Geology at ISU, took a class of 23 on a six-week driving tour of western Canada and Alaska. They traveled the nearly 14,000 miles in four Volkswagen minibuses. At that time more than 1200 miles of the Alaska Highway was dirt and gravel. During the course of the trip, the four vehicles sustained a blown engine, a transmission problem, a failed rear axle assembly, two broken windshields, and 23 flat tires! (Before the trip's end, the students became as adept at changing tires as an Indy 500 Pit Crew!) Despite problems with vehicles, by conducting field study of four biomes, it was a trip that was etched into everyone's memory for all time.

Overall, Dr. Jackson traveled nearly 100,000 miles with student groups, and discussed their observations around campfires in more than 40 states from Maine to Florida, and from Arizona to Alaska, during more than four decades of field study.

Then, after 38½ years of teaching at ISU, Marion was "retired" for only about 48 hours when St. Mary-of-the-Woods College asked him if he would consider coming there as a Visiting Professor of Ecology. He has been greatly enjoying teaching Earth Literacy Master's Students and undergraduates in biology at SMWC for over four years now!

HIS RESEARCH AND PUBLICATIONS

Marion has published a total of over 50 scientific publications, including several book chapters and two books. In addition, he edited Volumes 79–83 (1969–1973) of the *Proceedings* of the Indiana Academy of Science. He also has published an equal or greater number of semi-popular science articles, book forewords, newspaper features, essays in newsletters of environmental organizations, eulogies of colleagues, commentaries, poems, and other Science and Society communications. Many of his scientific publications are indicated in the bibliography that follows. A few of the research highlights of his career are indicated below.

He studied the effects of differences in microclimates on spring wildflower phenology at Allee Memorial Woods Nature Preserve, Parke County, Indiana, for his Ph.D. research (1961–1964). He co-authored a book chapter entitled "Plant Communities of Indiana" as Chapter 16 in *The Natural Features of Indiana*, with R.O. Petty of Wabash College (1966). In 1969, he published two book chapters on Hemmer Woods, Gibson County, and Potzger Woods, Ripley County, in *The Natural Areas of Indiana and Their Preservation.*

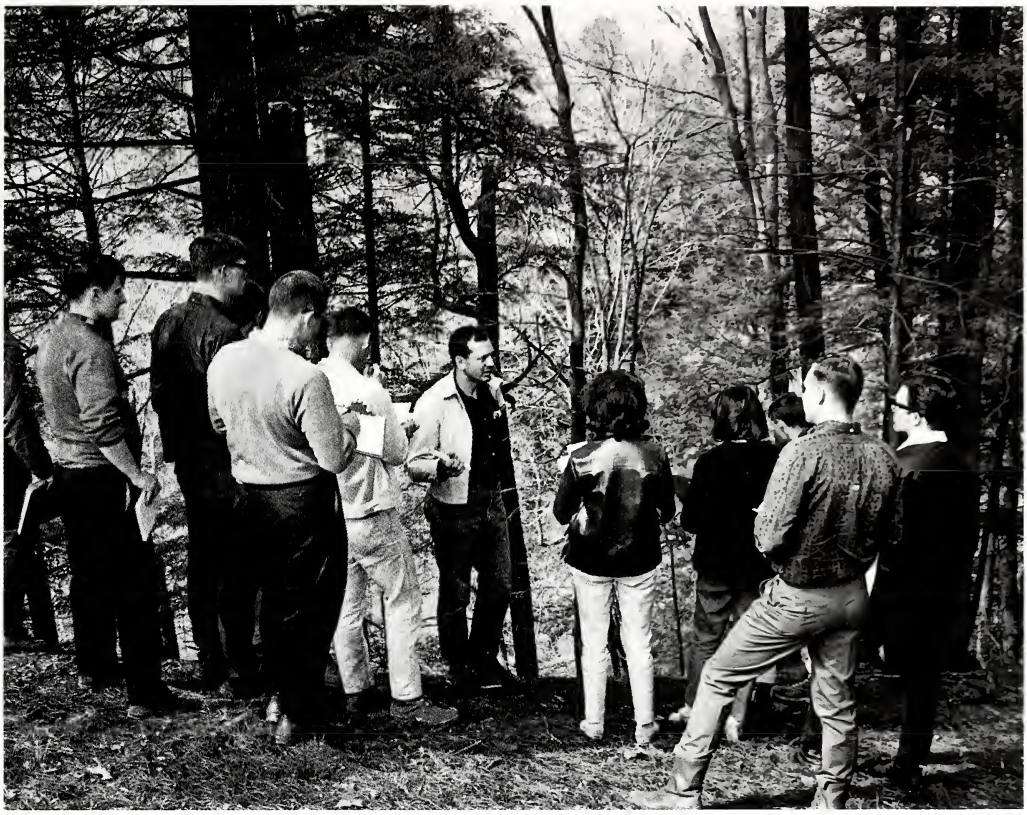

Figure 5.—Leading a plant ecology class at Indiana State University on a field trip to Pine Hills Natural Area, Montgomery County, Indiana (Spring 1967).

He and R.O. Petty completed an assessment of synthetic indices for evaluating forest importance values (1971). Jackson analyzed the vegetational ecology of an emperor goose nesting area in the Kokechik Bay Region of the Clarence Rhode National Wildlife Refuge, Alaska (1973).

He studied an old-growth forest at the prairie border in Edgar County, Illinois, as compared to GLO Survey Forest Composition, with Dr. M. Cowell, University of Missouri (2000–2002). Marion is still actively conducting re-surveys of vegetation studies originated early in his career. Dr. Mark Cowell, University of Missouri School of Forestry, Dr. Kem Badger, Ball State University, and former graduate students are assisting in these research studies.

Marion's research career has recently been capped by two clearly outstanding volumes. One is *The Natural Heritage of Indiana*, which he edited (Indiana University Press, 1997). This large-format, four-color book brings together 57 chapters on the Natural History of the State by many of the most knowledgeable scientists, and most gifted nature photographers, of Indiana. Marion contributed eleven chapters and many photographs himself. Few people other than Marion would have had the writing and editing skills, and much more importantly, the broad knowledge, along with the love of the State of Indiana, to do this project justice. And done it justice he has. It has received many accolades from near and far. It is a superb book, a beautiful book, and an outstanding source of information about our state.

The second book is *101 Trees of Indiana* (Indiana University Press, 2004), which is a beautifully illustrated field guide to all of the native trees of Indiana, plus coverage of more than 40 additional species that are widely encountered here. This, too, is a very complete

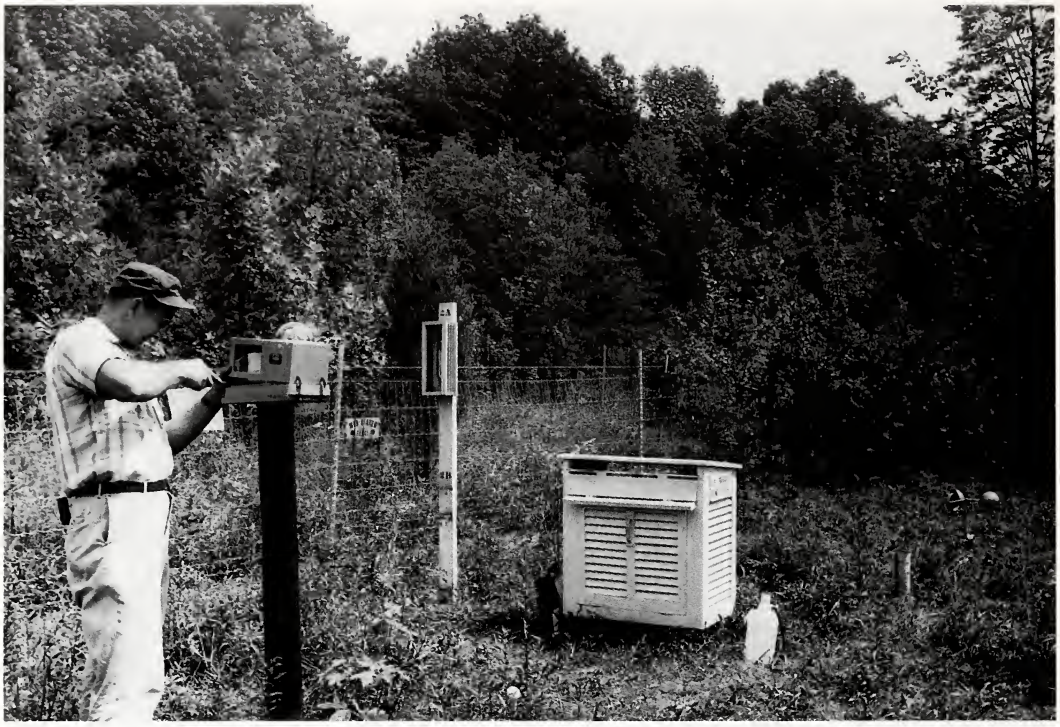

Figure 6.—Microclimatic field station with instrumentation in woody old field at Allee Woods, Parke County, Indiana (Spring 1963).

and beautiful volume which can only bring more admiration for his writing skills.

HIS STUDENTS

Fortunately, a number of the several thousand undergraduate students that Marion has taught were stimulated to continue in the fields of ecology or natural resource management, which is itself a measure of a master teacher. More than 40 students have completed the research requirement for their non-thesis M.S. degrees under his direction. An additional 18 M.S. and Ph.D. candidates completed their thesis or dissertation research under Dr. Jackson's mentorship. Much of his own work has been in close cooperation with graduate students.

Six of the 16 M.S. thesis students later received Ph.D. degrees in Plant Ecology from other institutions. Several of his other former M.S. degree students are now in practical or applied ecology positions such as state natural resource departments, and some are in teaching positions.

He and four of his M.S. Students (H. Donselman, C. Dunn, B. Hollett, and J. Levenson) studied the structure and dynamics of groundlayer, shrublayer, and canopy tree strata in 40 old-growth beech-maple stands in a five-state area (Indiana, Illinois, Michigan, Ohio, and Wisconsin) as part of an analysis of the beech-maple forest association (1973–1978).

Along with three M.S. students (E. Donselman, D. Kaminski, and L. Leitner) he analyzed GLO Survey Record data for presettlement forests in Vigo County, Indiana; the prairie border area of Indiana-Illinois; and in unglaciated southern Illinois (1974, 1978–79).

He and two M.S. students (K. Badger and L. McClain) described the ecological life histories of two tree species uncommon in Indiana, *Magnolia tripetala* and *Gymnocladus dioica* (1979, 1984).

Studies of the vegetation and floristics of two national parks were completed by Jackson and A. Faller on Wizard Island, Crater Lake National Park (1966–1969), and at Mammoth Cave National Park (1969–1973).

Old-growth forests in Jennings County, Indiana, and along the White River floodplain in Marion County, Indiana, were examined by

Jackson with W.B. Barnes and B. Hollett, respectively (1975, 1984).

Marion has conducted long-term ecological research on the structure and dynamics of a number of old-growth forests as follows: (1) Hoot Woods, Owen County, Indiana, for many years with four M.S. Students (B. Abrell, P. Allen, B. Morgan, and R. Strait), and with Dr. M. Cowell (1965–2005); (2) Dobbs Park Nature Preserve, Vigo County, Indiana, with M.S. students (N. Hoalst and R. Helms), and with Dr. M. Cowell (1975–2000); (3) Kramer Woods, Spencer County, Indiana with B. Abrell, and Dr. D. Schmelz (1992–2002); (4) Stout Grove, Jedediah Redwoods State Park, California with B. Abrell (1966–2006). Pre- and post-burn analyses and other studies were conducted at Little Bluestem Prairie Nature Preserve with three M.S. students (D. Dubis, M. Sheerer, and R. Strait) (1984–1989). The impact of different levels of trail use in Indiana Nature Preserves was examined with M.S. student, G. Adkison (1991–1996).

HIS CONSERVATION EFFORTS AND SERVICE

Dr. Jackson is one of the few who fully understands the adverse effects that we humans are having on our only home, Planet Earth; and throughout his academic career, he has been in a position to help people to decide what to do to correct these problems. He can vividly bring out the facts, lead students or environmental groups into drawing their own conclusions, and then develop solutions to problems. Marion has lived the life that he has taught his students to lead. He understands that many of the major problems of the world are environmental, and that they often dwarf all the other major problems of the world.

Marion is perennially a member of 20–30 environmental or conservation organizations, and has been quite actively involved with, or an officer in, a number of them. He holds Life Memberships in several of these.

Marion spent four summers working for the Federal Government in the American West: for the U.S. Forest Service in the St. Joe National Forest, at Moscow, Idaho, in forest fire detection and suppression in 1961; at Crater Lake National Park, Oregon, as a seasonal Interpretive Ranger-Naturalist in the summers of 1965 and 1966; and on the Clarence Rhode National Wildlife Refuge, Bethel, Alaska, surveying the nesting ecology of the emperor goose in wet tundra along the Bering Sea, in 1973.

In 1967, Marion gave testimony at hearings before the Indiana State Legislature urging passage of the Indiana Natural Areas Protection Act, the enabling legislation for the Indiana Nature Preserves System, and the Indiana Division of Nature Preserves, within the Indiana Dept. of Natural Resources.

During 1973–1974, he prepared evaluation reports for 28 Indiana Natural Areas being considered for National Natural Landmark status by the National Park Service. Following submission of Marion's reports, Indiana had more National Natural Landmarks than any other state.

Marion served on the Organization Committee when the Natural Areas Association was founded at a meeting in St. Louis during the late 1970s. He served on the Board of NAA for three years.

At Dr. Alton Lindsey's suggestion, Marion became a member of The Nature Conservancy in 1961, and became a Life Member in 1980. He served as President of the Indiana Chapter of The Nature Conservancy for seven years, 1975–1983. During that period, the Indiana chapter received the annual best chapter award for the U.S. on two occasions. Marion served as Acting State Director of the Indiana Chapter in 1978–1979, during State Director Dennis Wolkoff's sabbatical. He has been a Life Trustee of the Indiana Chapter for more than 20 years.

Marion has worked closely with William B. Barnes, Sr., James Keith and John A. Bacone, successive Directors of the Division of Nature Preserves, for more than 35 years toward the identification, protection, and management of natural areas throughout Indiana. During 1979–1980, while on sabbatical leave from ISU, Marion served as Program Ecologist for the Indiana Natural Heritage Protection Program during its formative year. During that time, he worked up a Land Classification System for the entire State of Indiana. He worked with Bill Barnes on the designation of Dobbs Park and Flesher Woods Nature Preserves in Vigo County, Hemmer Woods in Gibson County, Lubbe Woods in Dearborn County, Tribbett's and Commiskey Woods in Jennings County, and several other natural areas.

Marion also worked closely with The Na-

ture Conservancy, Indiana Chapter, and the Indiana State University Administration to protect three other Natural Areas in Vigo County (Little Bluestem Prairie, Kieweg Woods, and Ouweneel Woods) and guided the transfer of these tracts to ISU for use as teaching and research areas.

For many years, Marion has been actively involved with the Wabash Valley Audubon Society, serving as Vice President, President for three years, Environmental Service Award Selection Committee, and several other posts.

Marion served as an unpaid consultant to Pat Daugherty, Director of Terre Haute City Parks, on the development of the Dobbs Park Nature Center/Environmental Education Program during the early 1970s. His former M.S. student, Ronald Helms, became the first Interpretative Naturalist when the Nature Center was dedicated. For decades, he has worked with Keith Ruble, Director of the Vigo County Parks on the establishment, implementation and use of the county park system, and the development of recreational, educational and interpretive programs at the county parks. Marion has served for many years as an Honorary Trustee of ACRES Land Trust, Inc., a very successful Natural Areas protection group in northeastern Indiana.

Marion has been a member of the Indiana Academy of Science since he first entered graduate school in 1961. He was the first chair of the newly-formed Ecology Division, and chaired that division on at least one other occasion. He was also chair of the Plant Taxonomy Division (now Plant Systematics and Biodiversity). He and his students have presented at least 25 papers at annual meetings of IAS.

Marion served as Editor of the *Proceedings* of the Indiana Academy of Science for five years, 1969–1973. During his tenure as Editor, he instituted full peer-review as a prerequisite for all full-length submitted papers before acceptance for publication. He personally published at least 13 research papers, plus numerous abstracts, in the *Proceedings*.

Marion served as President-Elect of the Academy in 1998, when Dr. Rebecca Dolan was President, then served as President in 1999. He was a member of the Executive Committee for at least 10 years, plus service on the Publications, Biological Survey, Preservation of Natural Areas, and Membership committees for multiple years each. He served on the Program Committee for the Indiana State University Meetings in 1987, and was Co-Program Committee Chair, with Dr. Joyce Cadwallader, for the St. Mary-of-the-Woods College Meetings in 2005. He became a Fellow of the IAS in 1976.

For his service, teaching, and research, Marion has received numerous awards. Marion received a Certificate of Appreciation from the Indiana Conservation Council, Inc., in 1974 for Indiana Natural Areas work; and he was awarded a Certificate of Merit from the U.S. National Park Service in 1975 for National Landmark evaluations. The Nature Conservancy presented him their highest member recognition, the Oak Leaf Award, in 1981, for distinguished Natural Areas work in Indiana. The Wabash Valley Audubon Society granted him its James H. Mason Environmental Service Award in 1994.

Indiana State University has presented him a number of awards and recognitions. He received the Caleb Mills Distinguished Teaching Award in 1997; the College of Arts and Sciences Distinguished Professor Award in 1999; and the College of Arts and Sciences Medallion in 2000. His life and career were featured in the *Indiana State University Magazine* in its Summer 1998 issue, in an article entitled *A Natural Life*.

PRESENTATIONS

During his career, Marion has reviewed and/or edited a number of books, laboratory manuals, and popular articles, both before and after publication. He serves as Ecological Consultant for *Snowy Egret*, reported to be the nation's oldest nature magazine.

During his career, Marion has been a popular and effective invited speaker before groups ranging from national and state scientific and environmental organizations, to K-thru-12 school classes, civic organizations, Scout troops, church groups, etc. He has made literally hundreds of presentations during his career. For example, he has made at least 20 presentations on each of his two books.

"As the Twig is Bent"
Marion Jackson (2006)

An old proverb states, "As the twig is bent, so the tree shall grow." This is also true of

humans. As the mind is conditioned, so shall we think.

When a person nears the end of their career and enters the Autumn Years of one's life, it is only natural to become more reflective regarding the events that have made them *who* and *what* they are. This is especially true if one has the inquiring mind of a scientist.

For decades, I have been telling my students that the secret of success can be summed up in six words–namely, "*We become what we think about.*" When our life nears completion, perhaps what distills down from our human sojourn is *the sum total of our thoughts*, especially as our thoughts are translated into action.

But this admonition is a two-edged sword. Successful people think positive thoughts; those less so spend much of their time wallowing in negative thinking. It behooves one to choose their thoughts carefully, and to associate with positive thinkers who, themselves, are successful. You see the mind is a lot like fertile soil. Soil does not care what is planted in it, but will return what *is* planted in abundance—it will produce weeds or useful crops in equal measure. So will the human mind.

At this time, I should like to recognize those who have been most influential in shaping and guiding my lifelong thinking, and, as such, those who have given me a "leg up" toward whatever success I have achieved. In the words of Coleridge, "The dwarf sees farther than the giant, when he has the giant's shoulders to mount on."

Besides my parents, siblings, close relatives, neighbors, and friends who lived in the rural Tanglewood neighborhood of my early years, I need to mention certain teachers who were at the Versailles School during the 1940s and early '50s. Five emerge above the usual plateau of competence: Forest G. Waters, Principal, who represented fairness and firmness in education; Olive S. Smith, who excited me with the discipline of mathematics; Walter Phinney, a handicapped teacher on crutches who epitomized dedication and perseverance; Rex B. Davis, who taught me how to take organized class notes; and Edna Koehne, who taught me to read.

It was my extraordinary good fortune to encounter 14 professors at Purdue University who were among the finest scientists and educators of the Midwest: *Alton A. Lindsey (plant ecology); Durward L. Allen (wildlife ecology); *Howard Michaud (conservation education); Leland Chandler (insect ecology); Russell E. Mumford (wildlife ecology); Charles M. Kirkpatrick (wildlife management); *Arthur T. Guard (plant anatomy); *Samuel Postlethwaite (plant morphology); Grady L. Webster (plant taxonomy); James Ahlrichs (soils); *Wilton N. Melhorn (glacial geology); James E. Newman (bioclimatology); David C. "Pappy" Pfendler (Asst. Dean of Agriculture); and Paul B. Alexander (Dean, School of Education). Collectively, they provided the nucleus of both my undergraduate and graduate degrees.

Colleagues at Indiana State University who must be mentioned are: William J. Brett (Chair, Life Sciences Department); John O. Whitaker, Jr.; (vertebrate ecology); *Benjamin Moulton (Chair, Geography-Geology Department); *William B. Hopp (Chair, Science Division): Jack R. Munsee (entomology); and Marshall Parks (science education).

Co-workers in the field of natural area preservation include: William B. Barnes, Sr., and John A. Bacone (successive Directors, Division of Nature Preserves); Lynton K. Caldwell (first President, Indiana TNC); Dennis Wolkoff, and William Weeks (successive Directors, Indiana TNC); Paul Carmony (Registry Director, Indiana TNC); *Damian V. Schmelz (Natural Resources Commission); Robert C. Weber (ACRES Land Trust); and Robert O. Petty (Wabash College, and *my closest professional friend*). ("*" indicates former presidents of the Indiana Academy of Science).

For years, I have said that, "When I stop learning from my students, I will quit teaching."

Of the thousands of former students who have passed through my classes, W. Keith Ruble (Director, Vigo County Park Department) has earned my greatest admiration for his integrity, his accomplishments, and his dedication to protecting Indiana's historical heritage, and its natural resources. He has taught me so much.

And finally, I will mention the two books, (both of which I read during my freshman year at Purdue), that have influenced my thinking, and my career more than any dozen others, namely: Aldo Leopold's *A Sand County Almanac*, and Durward Allen's *Our Wildlife*

Legacy. These two men's understanding of how the natural world *should* work has become my second religion.

In sum, it has been a rich and most enjoyable career and life. When I see these highlights set down in one writing, it somehow seems impossible that all these things did indeed happen. But perhaps they did, and as old-time baseball pitcher, "Dizzy" Dean, once observed in his inimitable manner of speaking, "It ain't braggin' if you actually dun it."

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MINUTES OF THE MEETINGS OF THE INDIANA ACADEMY OF SCIENCE 2005

COMBINED MEETING OF THE EXECUTIVE COMMITTEE AND THE COUNCIL

St. Mary-of-the-Woods College
22 April 2005

EXECUTIVE COMMITTEE MEETING

2:00 pm

Attending.—Uwe Hansen, Ed Frazier, Jeff Hughes, Clare Chatot, Nils Johansen, Nelson Shaffer.

Hansen welcomed the Executive Committee. The purpose of the meeting was to briefly summarize the thinking behind some of the agenda items at the Council meeting.

Technical initiatives.—Hansen is very interested in re-vitalizing the Sections and also pulling the Section Chairs closer into the operations of the Academy. The individual sections will have available to them a small amount of money to add to the section activities at the Fall meeting. A suggested item would be a Section Distinguished Speaker, but it would be up to the individual sections to decide on this.

Section additions and name changes.—Hansen will propose the establishment of a Mathematics Section at the Council meeting and also propose to change the name of the Geology/Geography Section to the Earth Science Section to closer represent the many faces of the section: traditional geology and geography, but also astronomy and meteorology as two examples.

Hansen will announce a meeting of Section Chairs and/or their representatives for a brief meeting after the Council meeting to address the student award for presentations at the Fall meeting and the mechanics of picking the overall winners.

These items were approved by consensus and will be included at the Council meeting.

The Executive Committee Meeting was adjourned at 2:13 pm.

COUNCIL MEETING

2:30 PM

Attending.—Uwe J. Hansen (President), Clare Chatot (President-Elect), Edward Frazier (Treasurer), Nils I. Johansen (Secretary), Nelson Shaffer (Executive Director), Damian Schmelz (Natural Resources Commision), Marion T. Jackson (Past President), Holly Oster (Librarian), Daryl Karns (Ecology Section Chair), Jeff Hughes (Research Grants Chair), Jodie Ellis (Entomology Section Chair), Kimberly Lyle-Ippolito (Cell Biology Section Chair), Blake Janutolo (Botany Section Chair), Joyce V. Cadwallader (Saint Mary-of-the-Woods College), Marcia Moore (Website Manager), Jim Berry (Editor of the *Proceedings*), Mike Foos (Nominations Committee), Gary Moughler (Entomology Section Vice-chair), Liz Brown (Mathematics Section Chair), Robert F. Dale (Past Secretary), David Daniell (Invitations and Programs Committee), Rich Kjonaas (AAAS Representative and Chemistry Section Chair), William R. Gommel (Historian/Necrologist).

Welcome.—Hansen welcomed the council members at 2:30 pm.

Introductions.—The council members introduced themselves and their affiliations within the Academy.

Additions to the agenda.—Hansen asked for additions; there were none.

Approval of the agenda.—Hansen asked for approval of the agenda; unanimous approval.

Approval of minutes.—Nils Johansen passed out the minutes of the Council Meeting of 28 October 2004 at Hanover College for approval; unanimous approval.

INDIVIDUAL REPORTS

Treasurer.—Edward Frazier handed out the 2004 year end report and the January–April income and expense reports.

Executive Director.—Nelson Shaffer handed out an information letter about the Academy. He also said the Academy would

be represented at the Earth Day celebration in Indianapolis this weekend (23–24 April 2005). He also commented on the recent dues increase saying that there had not been any significant impact on the total membership in terms of numbers.

Editor of the *Proceedings*.—Jim Berry handed out a summary of the overall *Proceedings* activity over the last five years. Compared to last year, 9 manuscripts had been received by April 2005, compared to 4 the previous year over the same time span. He encouraged the sections to submit papers for publication.

Newsletter Editor.—Uwe Hansen (Don Ruch was not present) summarized the present status of the Newsletter and encouraged the members to submit newsworthy items to the Newsletter editor.

Secretary.—Nils Johansen had no additional items (the minutes had been distributed and approved earlier).

President-Elect.—Clare Chatot will organize and run the student award program at the upcoming Fall meeting.

President.—Uwe Hansen made a few general comments. He asked for a motion to approve a new Mathematics Section, Michael Foos made the motion, Oster seconded the motion, and there was unanimous approval. Liz Brown was appointed interim Mathematics Section Chair. Bill Gommel reminded the newsletter editor to include this (the new Mathematics Section and its interim chair) in the upcoming newsletter and encourage attendance at the Fall meeting. The Executive Committee's proposal to re-name the Geology/Geography section as the Earth Science Section was unanimously approved.

COMMITTEE REPORTS

Foundation.—No report, but Ed Frazier had earlier talked about the interactions with the Foundation.

Research grants.—Jeff Hughes summarized the status of the applications at this time. The committee will meet in Evansville next week to finalize the awards. Peter Scott is a new member of the committee.

Programs.—David Daniell said the 2006 meetings will be at Ball State; exact dates will be forthcoming.

Local arrangements.—Joyce Cadwallader and Marion Jackson said there will be a 12 May meeting of the Local Arrangements Committee. The call for papers will go in the Newsletter. Because of the early time of the Fall meeting (6–7 October), the abstracts will not be mailed out before the meeting, but will be available to be picked up at the meeting by attendees. After the meeting the remainder of the membership will get the abstract mailed to them.

Daryl Karns said that in connection with the Fall meeting, there would be a special section on the Heritage of the Wabash River. (An e-mail was sent out 25 April 2005 outlining the ideas for the special session). Thursday evening he plans to have a reception/dinner with a speaker; and on Friday morning there will be papers followed by Friday afternoon field trips. The details will be worked out.

Nominations.—Michael Foos reminded the Council about upcoming elections and the need for nominees. He specifically brought to the attention that among others, the Treasurer is up for election and also the annual President-Elect position. All the positions will be listed on the web-site, and he stressed the need for nominees for the positions.

Publications.—Nelson Shaffer gave an oral report for Bill McKnight summarizing the current activities.

Finance.—Edward Frazier (Ed Squires was not present) summarized the activities of the Finance Committee.

Resolutions.—Nelson Shaffer reminded the local organizing committee of the resolutions normally presented at Fall meetings and the need to have them submitted to him before the Fall meeting.

Library and archivist.—Holly Oster gave an oral report on the status of the library.

Amendments.—No report.

Youth activities.—Zeck (not present) submitted a written report.

Awards.—Clare Chatot reminded the Council to encourage members to nominate deserving members for Fellow of the Academy.

Biodiversity.—No report.

Junior academy.—No report.

Talent search.—No report.

Web.—Marcia Moore submitted a written report. She highlighted the "Registry of Experts" efforts and also—with Uwe Hansen—the work on section news on the web-site.

Speaker of the Year.—No report.

Natural resources.—Damian Schmelz submitted a written report.

AAAS representative.—Rich Kjonaas submitted a written report. Discussion followed on the current status of teaching of evolution *vs.* creationism or intelligent design. Should the Academy again raise the issue in Indiana as was done at the 1999 meeting? Damian Schmelz reminded the Council to be aware of the quality of "scholarship" on the part of the creation science community. Also, this debate should include the Junior Academy. No votes were taken.

Historian/Necrologist.—Bill Gommel reported the passing of two members: Dr. Herbert C. Brown (the recipient of the 1979 Nobel Prize in Chemistry) and Dr. William J. Rowland.

Meeting site for 2006.—See "Programs" above.

Unfinished business.—None.

New business.—None.

The meeting was adjourned at 4:22 PM.

SECTION CHAIR MEETING
5:00 PM

This was an information meeting. President Hansen addressed the Section Chairs, Section representatives and other interested members of the Council where he again expressed his vision for Section re-activation. He stated that the sections could apply for funding for the specific section activities from a $2000 fund in the operating budget, or about $200 for a given section. Some Section Chairs indicated that they would be interested in using some of these funds for the Fall meeting. Hansen would be notified over the summer.

The meeting was adjourned at 5:30 PM.

Respectfully submitted,
Nils I. Johansen, P.E.
Secretary

MEETING OF THE
EXECUTIVE COMMITTEE
AND THE COUNCIL
St. Mary-of-the-Woods College
St. Mary-of-the-Woods, Indiana
6 October 2005

EXECUTIVE COMMITTEE MEETING

Welcome.—The meeting opened at 1:37 PM with President Uwe Hansen presiding.

Organizational conference items.—Uwe Hansen expressed thanks to Marion Jackson and Joyve Cadwalleder for the local arrangements and for the abstract booklet that is available. Nelson Shaffer and Edward Frazier will make sure that the attendees will get one copy each and that non-attending members will get one mailed to them.

Technical initiatives.—President Hansen reported that three sections have started this project in their sections.

Wabash River symposium.—Daryl Karns gave some organizational information. About 70 people are expected to attend.

Other satellite organizational meetings.—None.

Items for the Council meeting.—President Hansen will encourage all sections to find their "movers and shakers" and will also mention this to Section Chairs. It is vital for the Academy that the sections are active. This is where the "action is" in the Academy.

Other items.—We need to identify future Fellows.

Nelson Shaffer indicated that Academy members should be aware as to "how to talk to the media." There was discussion regarding this topic as a future workshop. The annual Budget meeting will be at Butler University at 1 PM (Indianapolis time) on 10 December.

Adjournment.—The meeting adjourned at 1:55 PM.

COUNCIL MEETING

Attending.—Uwe Hansen (President), Clare Chatot (President-Elect), Edward Frazier (Treasurer), Nils I. Johansen (Secretary), Donald Ruch (Past-President), Nelson Shaffer (Executive Director), Marion Jackson (Program Co-Chair), Joyce Cadwallader (Program Co-Chair), Frank Guthrie (Academy Foundation), Jim Berry (Editor of the *Proceedings*), Jeff Marshall (Youth Activities), Patty Zeck (Youth Activities), David Daniell (Programs and Invitations Chair), Melissa Mitchell (Science Education Chair), Dan Webster (Past-President), Holly Oster (Librarian), Blake Januto lo (Botany Section Chair), Kimberly Lyle-Ippolito (Cell Biology Section Chair), Marcia Moore (Web-site), Michael Foos (Elections Committee Chair), Richard Kjonaas (AAAS Representative), John Shutt (Speaker of the Year Committee), and William R. Gommel (Historian/Necrologist).

Welcome.—President Uwe Hansen presid-

ed and welcomed the guests, and all attendees introduced themselves. The meeting opened at 2:06 PM.

Additions to the agenda.—Uwe Hansen expressed thanks to the people who worked hard on getting the program together and expressed appreciation to each.

Approval of agenda.—Uwe Hansen moved, Ruch seconded, to approve the printed agenda. There was unanimous approval.

Approval of Minutes from Spring 2005 meeting.—Nils Johansen handed out the minutes for approval. Ruch so moved, Chatot seconded. The minutes were approved.

INDIVIDUAL REPORTS

Treasurer.—Edward Frazier handed out a summary of our financial status and went through the report line by line. No action was taken.

Executive Director.—Nelson Shaffer handed out membership data. He also suggested the need to increase corporate membership. No action was taken.

Editor of the *Proceedings*.—Jim Berry handed out his report. Biographies of distinguished IAS scientists will be a new feature in the *Proceedings*. It will start with the next issue.

Newsletter Editor.—Don Ruch said the Newsletter is "doing fine." He reminded members that the deadline for the last issue in 2005 is 15 November, and he encouraged members to submit articles to "let people know what the IAS is doing."

Secretary.—Nils Johansen had no additional items to his earlier report (above).

President-Elect.—Clare Chatot said that she will run the Student Presentation Competition. She will meet with the Section Chairs at lunch regarding this; and, also, she will coordinate the Technical Initiatives Program for next year and also the Abstract submission. She announced that the Budget Committee meeting would be held at Butler University 10 December at 1 PM (Indianapolis time) and encouraged submissions from within the Academy for consideration to be included in next year's budget.

President.—Uwe Hansen reiterated what he had said in the Executive Committee meeting. We have the "technical initiatives." Also, we should recognize "movers and shakers" of the Academy at various institutions of higher learning within the State. Section Chairs could take this on as a Section project, thus getting a historical, biographical bird's eye view of what has happened to and within the Academy over the years.

COMMITTEE REPORTS

Foundation.—Frank Guthrie gave a few comments on his written report.

Research grants.—Nelson Shaffer commented on the written report.

Programs.—David Daniell reported that the 2006 meetings will be at Ball State and that the 2007 meetings probably at the University of Indianapolis. There was a comment from the floor about a future meeting at IU or Purdue. The answer was that these meeting are usually money-losing propositions for the Academy; however, presence at the "flagship" institutions of the state every 10 years or so might be worth looking into in terms of IAS exposure.

Local arrangements.—Joyce Cadwallader and Marion Jackson gave "nuts and bolts" information about the upcoming general meetings.

Nominations.—Michael Foos reported the results of the elections. They will be announced at the general membership meeting on the next day. The new President-Elect is John Schutt, Treasurer is Ed Frazier, Foundation representative is Frank Guthrie, and the member of the Research Grants Committee is Sandra Brake. A change in By-laws to reflect current election policies was referred to New Business.

Publications.—Edward Frazier presented a brief report.

Finance.—No report.

Resolutions.—Nelson Shaffer presented the resolution that will be read during the general membership meeting thanking St. Mary-of-the-Woods College for hosting the meeting.

Library and archivist.—Holly Oster passed out her report. She also mentioned that she needs member biographies and also pictures of some Past-Presidents.

Amendments.—There were no amendments at this time.

Youth activities.—Patty Zeck made a report. She and Jeff Marshall are co-chairs. She shared several stories about the successes of

the program. She is open to suggestions on future needs. Don Ruch added his personal thanks to Patty and Jeff Marshall for their work.

Awards.—Clare Chatot reported that there were no Fellow nominations this year. She charged the Section Chairs with finding and nominating worthy members. Don Ruch will also put a reminder in the newsletter.

Biodiversity.—Don Ruch reported that, as part of the Wabash River Symposium, there would be a 2–4 PM mini bio-blitz with 32 participants. Daryl Karns also informed the members about the symposium.

Junior Academy.—This was discussed under Youth Activities (above).

Talent search.—This was discussed Youth Activities (above).

Web-site.—Marcia Moore gave an oral report. President Hansen and Don Ruch expressed their appreciation to Marcia for putting the program on the web-site.

Speaker of the Year.—John Schutt announced that Michael J. Lannoo would be the luncheon speaker and the title would be "Insights Into Causes of Malformed Frogs." Also, if you want the Speaker of the Year to come to your institution, contact John Schutt.

Natural resources.—A written report was passed out.

AAAS.—Richard Kjonaas passed out a report.

Historian.—Bill Gommel reported the passing of the following members: Herbert C. Brown, Alice R. Copess, William J. Rowland, Joseph R. Siefker, and Helene Starcs. He will also announce this at the annual lunch meeting.

Unfinished business.—Micheal Foos' suggestion on format for nominations and possible by-law changes in this regard will be forwarded to Bob Waltz to be discussed in his committee.

New business.—Patty Zeck indicated the need for IAS to be involved with the 2006 Science Fair. She will take it up at the December budget meeting.

A question was raised about an IAS registry of expertise and also preservation of IAS heritage. No decisions were made. Uwe Hansen and others will look into this.

Adjournment.—The meeting adjourned at 4:20 PM.

ANNUAL BUSINESS MEETING
St. Mary-of-the-Woods College
St. Mary-of-the-Woods, Indiana
6 October 2005

President Uwe Hansen presided. John Schutt introduced the Speaker of the Year, Dr. Michael J. Lannoo, who gave the talk "Insight Into Causes of Malformed Frogs." It was an excellent talk on the multi-faceted assault of people on an ecosystem and the results as shown in and by frogs. Both chemicals and parasites can cause malformed frogs; and, furthermore, both of these vectors can be traced to human activities. President Hansen read the resolution thanking St. Mary-of-the-Woods College for hosting the meeting and the Local Arrangements Committee for a job well done.

Micheal Foos reported the election results. The President-Elect is John Schutt, the Treasurer is Ed Frazier, the member of the Academy Foundation is Frank Guthrie, and the member of the Research Grants Committee is Sandra Brake.

Bill Gommel read the names of members who have passed since last year, and they were honored with a moment of silence.

President Hansen thanked the members of the Academy for the support he had received as President, and he especially thanked his predecessor, Don Ruch. He also charged the Section Chairs to discover people in their sections who have made an impact on IAS. As his last act as President, he passed the gavel to incoming President, Clare Chatot.

Respectfully submitted,
Nils I. Johansen, P.E.
Secretary

BUDGET MEETING
Butler University
10 December 2005

Attending.—Clare Chatot (President-Elect), Edward Frazier (Treasurer), Nils I. Johansen (Secretary), Donald Ruch (Past-President), Nelson Shaffer (Executive Director), Frank Guthrie (Academy Foundation), Jeff Marshall (Youth Activities), Patty Zeck (Youth Activities), David Daniell (Programs and Invitations Committee), Jim Bandoli (Research Grants), Bill McKnight (Publications Committee).

President-Elect Clare Chatot presided. Edward Frazier went through the 2005 and 2006

budgets. About $6000 is needed from the Foundation to balance the 2005 budget. The 2006 budget is expected to come out even. The 2006 budget as proposed by Frazier, and with minor changes, was unanimously approved.

The meeting was adjourned at 2:12 PM.

EXECUTIVE COMMITTEE MEETING
Butler University
10 December 2005

Attending.—Clare Chatot, Don Ruch, Jim Bandoli, Nelson Shaffer, Edward Frazier, and Nils Johansen.

Edward Frazier moved, seconded by Ruch, unanimous approval, that the Treasurer be authorized to sign checks to: Jim Berry, $4000 as Editor of the *Proceedings*, Bill McKnight, $4000 as Chair of Publications, and to Marcia Moore, $2000 as Webmaster.

Two informational issues were discussed: suggestions for publication inventory reduction, and the assembly of an IAS committee to look into the Intelligent Design/Evolution controversy. No conclusions were reached.

Respectfully submitted,
Nils I. Johansen, P.E.
Secretary

INDIANA ACADEMY OF SCIENCE
2005 Year End Financial Report

	Balance 1-Jan-05	Revenues	Expenses	Balance 31-Dec-05
OPERATING FUND				
Dues		22,565.00		
Interest		860.80		
Contributions		170.00		
Spring Meeting		455.00	500.11	
Annual Meeting		8,626.00	8,989.68	
Transfer from IAS Foundation		7,000.00		
Misc. Income		771.50		
Officer's Expenses			4,000.00	
Operating Expenses			8,616.88	
Financial Expenses			3,202.78	
Newsletter Expenses			2,280.80	
Library Operations			0.00	
Committee Expenses			0.00	
Speaker of the Year			700.00	
AAAS Representative			875.00	
Web Site Development			2,074.97	
Youth Activities			5,766.77	
Operating Fund Total	9,771.06	40,448.30	37,006.99	13,212.37
RESTRICTED FUNDS				
Proceedings	15,616.24	10,141.37	15,966.48	9,791.13
Publications	19,520.96	6,005.05	9,826.71	15,699.30
* Research Grants	16,027.13	72,481.27	72,544.91	15,963.49
Indiana Biological Survey	(515.58)	0.00	0.00	(515.58)
Lilly Library	6,578.44	0.00	0.00	6,578.44
Welch Fund	8,706.01	368.49	400.00	8,674.50
Life Members Fund	4,795.25	1,500.00	0.00	6,295.25
Past Presidents Fund	9,178.39	0.00	400.00	8,778.39
Total Restricted Funds	79,906.84	90,496.18	99,138.10	71,264.92
Prepaid Dues	4,375.00			5,130.00
TOTAL FUNDS	**94,052.90**	**130,944.48**	**136,145.09**	**89,607.29**
FUNDS ON DEPOSIT				
Checking Account	3,544.02	135,854.44	136,300.94	3,097.52
Money Market Savings	79,328.69	80,700.80	85,068.40	74,961.09
Cert. of Deposit	11,180.19	368.49	0.00	11,548.68
TOTAL FUNDS DEPOSITED	**94,052.90**	**216,923.73**	**221,369.34**	**89,607.29**

* Provided 27 senior member grants and 18 high school grants.

ACADEMY FOUNDATION FUNDS		
John S. Wright Fund Account	6,443,101.13	6,502,663.84
Academy Fund Account	242,405.60	242,560.75
Invested Income Account	384,885.45	495,283.84
TOTAL FOUNDATION FUNDS	**7,070,392.18**	**7,240,508.43**

Foundation Funded Used For:	
General Fund	7,000.00
Proceedings	9,248.87
Publications	0.00
Research Grants	63,990.19
Total	**80,239.06**

Edward L. Frazier
Treasurer

INSTRUCTIONS TO AUTHORS

(revised July 2005)

General information.—Manuscripts and all correspondence should be sent to the Editor. To be eligible for publication in the *Proceedings*, at least one author must be a member of the Academy. Papers that have been presented at an Academy meeting may be given preference for publication. Submission of a manuscript to the Editor implies that it has not been sent to another publication, nor has it been published elsewhere. If it is accepted for publication in the Proceedings, it may not be republished without the written consent of the Academy.

All manuscripts are evaluated by referees. Submit three copies of the manuscript. Figures in manuscripts for review may be submitted as photocopies, and they should be approximately the size that they will be printed in the *Proceedings*.

After the manuscript has been accepted, the author will be asked to submit the manuscript as a paper copy and on a computer disc in a widely-used word processing program. Indicate clearly on the computer disc the **word processing program** and the **type of computer** (Mac or PC).

Galley proofs will be sent to the primary author for approval and correction.

Voucher specimens and permits: Voucher specimens of species used in scientific research should be deposited in a recognized scientific institution. Authors must indicate that the collection of specimens, especially of threatened or endangered species, was authorized by the appropriate governmental organizations. All type specimens must be deposited in a recognized museum collection.

Style.—Do not use right or full justification. Also, use a non-proportional font (e.g, Courier, but not Times Roman).

Title page.—The title page should include (1) the title in capital letters, (2) each author's name and address, (3) the running head (see below), and (4) the complete name, address, and telephone number of the author with whom proofs and correspondence should be exchanged, a FAX number and electronic mail address if available.

Abstract.—All manuscripts have an abstract, which should summarize the significant facts in the manuscript. The "ABSTRACT" heading in capital letters should be placed at the beginning of the first paragraph set off by a period. Use complete sentences, and limit the abstract to one paragraph and 250 words.

Keywords.—Give 3–5 appropriate keywords following the abstract.

Text.—Double-space text, tables, legends, etc. throughout. Three categories of headings are used. The first category (METHODS, RESULTS, etc.) is typed in capitals, centered, and on a separate line. The second (lower) category of heading, in bold type, begins a paragraph with an indent and is separated from the text by a period and a dash.(This paragraph begins with an example of this heading.) The third heading category may or may not begin a paragraph, is italicized and followed by a colon. (The paragraph below is an example.) Use only the metric system unless quoting text or referencing collection data.

Citation of references in the text: Cite only papers already published or in press. Include within parentheses the surname of the author followed by the date of publication. A comma separates multiple citations by the same author(s) and a semicolon separates citations by different authors, e.g., (Smith 1990), (Jones 1988; Smith 1993), (Smith 1986, 1987; Smith & Jones 1989; Jones et al. 1990).

Literature cited section.—Use the following style, and include the full unabbreviated journal title. Repeat the name for multiple references by the same author. Note that book titles have the first letter of each word capitalized.

Walter, J. & B. Hallet. 1979. Geometry of former subglacial water channels and cavities. Journal of Glaciology 23:335–346.

Walter, J. 1992. The significance and complexity of communication in moths. Pp. 25–66, *In* Insect Communications: Mechanisms and Ecological Significance. (P.N. Work & J.S. Rivers, eds.). Princeton University Press, Princeton, New Jersey.

Footnotes.—Footnotes are permitted only on the first printed page to indicate current address or other information concerning the author. These are placed together on a separate page at the end of the manuscript. Tables and figures may not have footnotes.

Running head.—The author's surname(s) and an abbreviated title should be typed all in capital letters and must not exceed 60 characters and spaces. The running head should be placed near the top of the title page.

Tables.—These should be typed double-spaced, one table to a page and numbered consecutively. Most tables contain only three horizontal lines (see recent issues for examples). Do not use vertical lines or shading. Include all pertinent information in the table legend (no footnotes).

Illustrations.—All art work must be camera-ready (mounted and labeled) for reproduction. Figures should be arranged so that they fit (vertically and horizontally) the printed journal page, either one column or two columns, with a minimum of wasted space. When reductions are to be made by the printer, pay particular attention to width of lines and size of lettering in line drawings. Multiple photos assembled into a single plate should be mounted with only a minimum of space separating them. In the case of multiple illustrations mounted together, each illustration must be numbered Fig. 1, Fig. 2, etc.) rather than given letter (A,B, etc.) designations. The name(s) of author(s), and an indication of top edge, and whether the illustration should be one or two columns wide, should be written on the back of the illustration. The overall dimensions should be no more than 11 inches (28 cm) × 14 inches (36 cm). Larger drawings present greater difficulty in shipping and greater risk of damage for which the *Proceedings* assumes no responsibility. In manuscripts for review, photocopies are acceptable, and should be reduced to the exact measurements that the author prefers in the final publication. Make notations in the text margins to indicate the preferred position of illustrations in the printed text. Color plates can be printed; but the author must assume the full cost, currently about $600 per color plate.

Legends for illustrations should be placed together on the same page(s) and separate from the illustrations. Each plate must have only one legend, as indicated below:

Figures 1–4.—Right chelicerae of species of *Centruroides* from Timbuktu. 1. Dorsal view; 2. Prolateral view of moveable finger; 3. *Centruroides* holotype male; 4. *Centruroides* male. Scale = 1.0 mm.

Page charges and reprints.—There are no page charges for members of the Academy. Corrections in proof pages must be restricted to printer's errors only; all other alterations will be charged to the author (currently $3 per line).

Reprints are available only from Allen Press and should be ordered (with payment) when the author receives the proof pages. Allen Press will not accept reprint orders after the paper is published.

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